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# **Detection of an Invasive Aquatic Species by Canine Olfaction**

A thesis

submitted in partial-fulfilment

of the requirements for the degree

of

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at

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by

**JESSE AUSTIN QUAIFE**



THE UNIVERSITY OF  
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# Abstract

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Invasive species represent a major concern for native flora and fauna in New Zealand waterways. Current surveying methods employed in the detection of these species typically rely on visually observing or catching fish and are often expensive and difficult to implement. Given that freshwater fish release organic materials into the water, and that some of these materials contain volatile elements that are then released into the air above the surface, it was hypothesised that domestic dogs (*Canis familiaris*) may be able to detect the presence of these species by smelling water samples that have contained them.

In this study, four experiments were conducted to determine the validity of this hypothesis. Five pet dogs were trained using a go/no-go procedure to operate an automated apparatus that presented individual water samples through an opening in the front panel. Dogs were presented with samples from aquaria that had or had not contained fish.

In Experiment 1, dogs were presented with water from aquaria containing koi carp (*Cyprinus carpio*) and aquaria containing no fish. In Experiment 2, koi carp samples were systematically diluted until concentrations similar to those found within the natural environment were reached. Experiment 3 sought to determine whether dogs could discriminate koi carp from a distantly-related fish (brown bullhead catfish, *Ameiurus nebulosus*). Experiment 4 replicated the previous experiment with a closely-related fish (goldfish, *Carassius auratus*).

In all four experiments, dogs were able to correctly identify water that had contained koi carp and largely ignore water samples that had contained either no fish or other species of fish at above 80% accuracy. The overall results of this research indicate that dogs are able to accurately detect the presence of koi carp from water samples at concentrations similar to those found within the natural environment, and to discriminate between at least three species of fish. These findings suggest that dogs may have an important role to play in waterway conservation and management.

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# Chapter One

## Introduction

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### 1.1 Biodiversity decline

Global biodiversity is declining at an alarming rate (Pegas, Grignon, & Morrison, 2015; Sala et al., 2000). Conservative estimates suggest that the current rate of species extinction is now around 1,000 times higher than the natural rate; a figure that is projected to increase ten-fold in the near future due to complex changes in the environment (De Vos, Joppa, Gittleman, Stephens, & Pimm, 2015; Sala et al., 2000). This accelerated decline in biodiversity was recently recognised by the United Nations General Assembly who adopted resolution 65/161, declaring the years 2011-2020 the Decade for Biodiversity (United Nations, 2011). However, while the number of species threatened with extinction has risen considerably, the allocation of resources to alleviate this threat has remained relatively stable. That is, current threats to biodiversity worldwide far exceed available conservation resources (Myers, Mittermeier, Mittermeier, da Fonseca, & Kent, 2000; Welsh, Mohr, & Boase, 2017). Given this rather unsettling fact, conservationists must direct their efforts towards addressing the most pressing environmental concerns by identifying those species most at risk.

Of all the world's diverse biological ecosystems, freshwater habitats are perhaps the most endangered (Dudgeon et al., 2006); a far greater rate of biodiversity decline has been observed in fresh waters than similarly affected marine or terrestrial ecosystems (Sala et al., 2000). While freshwater habitats

comprise only 0.01% of the world's water (Gleick, 1996), they also support around 6% of the world's species and are home to as many as one-third of all vertebrate species (Dudgeon et al., 2006). The disproportionate richness of wildlife in these areas means that any changes to the natural environment can produce cascade effects throughout the entire ecosystem (Daborn et al., 1993; Dunne, Williams, & Martinez, 2002; Van Colen et al., 2015).

## **1.2 Biodiversity and the introduction of invasive species**

One driver of ecological change is the introduction of exotic species (Sala et al., 2000; Simberloff et al., 2013; Zambrano, Scheffer, & Martinez-Ramos, 2001). Although not all non-native species are considered invasive in their recipient habitats (Blackburn et al., 2011), only a minority are required to produce deleterious effects that are often difficult to reverse (Chan & Briski, 2017). These effects can have serious implications for conservation, extending not only to the destruction and depletion of natural resources, but also to the decline and extinction of endemic species (Simberloff et al., 2013).

One way to prioritise the allocation of conservation resources is to identify biodiversity 'hotspots'. According to Myers et al. (2000), these are areas in which "exceptional concentrations of endemic species are undergoing exceptional loss of habitat" (p. 853). According to this definition, New Zealand is one of 25 global biodiversity hotspots. As a remote island landmass, New Zealand has been fortunate to escape the invasion of a number of dangerous exotic species that plague the waters of other territories (Collier & Grainger, 2015). However, New Zealand is still recognised as one of six areas in the world in which an exceptional

variety of non-native fish have been introduced (Leprieur, Beauchard, Blanchet, Oberdorff, & Brosse, 2008). According to recent sources, around 43 freshwater fishes are native to New Zealand (McQueen & Morris, 2015), with an additional 21 having been introduced from overseas (Collier & Grainger, 2015). While not all of these species are considered pests, several have been classified as “noxious fish” (New Zealand Government, 1993) and “unwanted organisms” (New Zealand Government, 1983), and are associated with dramatic declines in water quality and biological diversity (Collier & Grainger, 2015). While it is difficult to untangle the complex causes of biodiversity loss, these “noxious” and “unwanted” fish have likely contributed to the decline of native and non-native fish species, of which 40 are now classified as either “threatened” or “at risk” (Goodman et al., 2014).

### **1.3 Koi carp as an invasive fish**

An invasive fish of particular concern is the non-indigenous fish koi carp (*Cyprinus carpio*), which arrived in New Zealand sometime in the 1960s (McDowall, 1990). Although the negative effects of koi carp have been well-documented since the early 1900s (e.g., Cahn, 1929), no significant concerns were raised in New Zealand until 1983 when self-sustaining populations were discovered breeding in the Waikato river (Tempero, Ling, Hicks, & Osborne, 2006).

Koi are colour aberrations of wild carp, resembling goldfish with their large, brightly-coloured scales (Balon, 1995). They can be distinguished from other species by two small whisker-like feelers (or barbels) at each side of the mouth, and are often marked by bright blotches of red, orange, black, white, or gold, with darker colours appearing along the dorsal and lateral areas (Collier & Grainger,

2015; McDowall, 2000). These vibrant fish are often referred to as 'swimming jewels' or 'swimming flowers', and are highly regarded in many parts of Eastern Asia, particularly in Japan where they have been selectively bred for their ornamental qualities for centuries (Balon, 1995; McDowall, 1990).

Although other non-indigenous fish species claim legal status as either "noxious fish" or "unwanted organisms", koi carp can be found among both of these categories for the destructive effect that they have on New Zealand waterways. Indeed, one author commented that koi carp are "without doubt, the least desirable fish in the New Zealand freshwater fish fauna" (McDowall, 1990, p. 235). One of koi carp's less admirable qualities is their ability to drastically modify the form and function of freshwater habitats (Cahn, 1929; Huser, Bajer, Chizinski, & Sorensen, 2016; King & Hunt, 1967). As omnivores, koi carp feed on benthic invertebrates and other planktonic organisms found along the beds of rivers and lakes (Fischer, Krogman, & Quist, 2013), occasionally also consuming submerged plant seeds and macrophytes (Crivelli, 1983; King & Hunt, 1967; Miller & Crowl, 2006). The absence of teeth precludes any direct predatory behaviour towards other fish (Sibbing, 1988), however, it is the indirect effects of koi carp's feeding behaviour that remain a cause for concern. During foraging, for instance, koi carp suction organic materials through the sediment and expel the remaining matter into the water (Collier & Grainger, 2015). This distinctive feeding pattern physically uproots submerged vegetation and suspends sediment and algal cells in the water column (Roozen et al., 2007), resulting in an increased availability of nutrients that leads to algal blooms and reduced water clarity (Badiou & Goldsborough, 2010; Fischer et al., 2013). This nutrient-rich water is less transparent, obstructing light

penetration below the surface and affecting the growth of submerged macrophytes (Crowder & Painter, 1991; de Winton, Drugdale, & Clayton, 2001). The presence of carp in large numbers has been linked to reduced water clarity (Badiou & Goldsborough, 2010; Chumchal, Nowlin, & Drenner, 2005; Jackson, Quist, Downing, & Larscheid, 2010; Rowe, 2007), and increased water turbidity (Akhurst, Jones, Clark, & Reichelt-Brushett, 2012; Lougheed, Theysmeyer, Smith, & Chow-Fraser, 2004). High densities of carp are also associated with a lower abundance and diversity of underwater vegetation (Badiou & Goldsborough, 2010; Bajer, Sullivan, & Sorensen, 2009; Crivelli, 1983; Jackson et al., 2010; Miller & Crowl, 2006), sport fishes (Jackson et al., 2010), and waterfowl (Bajer et al., 2009). Some of these data are correlative, however, a series of studies in experimental ponds have documented the direct trophic effects that these fish have on their recipient habitats (e.g., Badiou & Goldsborough, 2010; Barton, Kelton, & Eedy, 2000; Crivelli, 1983; Driver, Closs, & Koen, 2005; Huser et al., 2016; Kloskowski, 2011; Lougheed et al., 2004; Roberts, Chick, Oswald, & Thompson, 1995). Scheffer, Hosper, Meijer, Moss, and Jeppesen (1993) note that the combination of these effects results in a shift of physiochemical states (i.e., from a macrophyte- to plankton-dominated equilibrium), which can drastically modify the topography and function of freshwater habitats and drive out certain species that reside there.

Unlike other more delicate species of fish, koi carp are tolerant of a wide range of environmental stressors and habitat variations, including highly turbid waters, low oxygen conditions (less than 15% saturation; Hicks & Ling, 2015), and extended periods of starvation (Balon, 1995; McDowall, 1990). They can be found

anywhere from ice-covered lakes to tropical rivers (4-35°C; Bajer, Chizinski, & Sorensen, 2011; McDowall, 1990), and have been discovered living in brackish and highly saline waters (Balon, 1995), and in salinities of up to 14% for short durations (Hicks & Ling, 2015).

In addition to their resilience, koi carp are capable of covering extensive terrain within rivers and tributaries. Daniel, Hicks, Ling, and David (2011) found that koi carp in the Waikato River travelled a mean distance of 39 km over a 250-day period, with 74% of those monitored moving between the Waikato River and lateral habitats. Stuart and Jones (2006) followed the movements of koi carp in an Australian river and found that while most (66%) fish were recaptured within 1 km of their release location, several covered distances of over 200 km upstream, and one was observed travelling 890 km downstream over a period of 1,107 days. Although most koi carp demonstrate high site fidelity, their ability to migrate is concerning given their impact on natural ecosystems and the consequences this can have across multiple freshwater habitats.

The issue of migration is compounded further by the koi carp's high fecundity. A female of 6 kg has been known produce as many as 1.5 million mature oocytes, and is capable of multiple spawnings per season (McDowall, 1990). Tempero et al. (2006) found that female carp living in the Waikato River had a mean total fecundity of 299,000 oocytes, with an average of 97,200 oocytes per kg of bodyweight. This high fertility rate makes attempts at population control exceptionally difficult.

Like all invasive species, koi carp represent an environmental concern only once they exist above a certain biomass density. Recent evidence suggests that koi carp contribute to significant ecosystem degradation in shallow eutrophic lakes when biomass density levels exceed 50-100 kg/ha (Badiou & Goldsborough, 2010; Bajer et al., 2009). At some locations in the Waikato, koi carp occur at densities of up to 2,000 kg/ha and account for some 95% of the total fish biomass in these areas (Osborne, 2006). These rather startling statistics suggest that koi carp are causing significant damage to the ecological integrity of freshwater habitats in the Waikato region.

So how does one go about controlling or attempting to eradicate a fish that is omnivorous, highly fecund, migratory, and tolerant of a range of environmental stressors? With most approaches, the first step is detection.

## **1.4 A review of popular fish survey methods**

A number of techniques are commonly employed in the detection of invasive fish (Grainger, West, & McCaughan, 2014). While some of these techniques involve the use of off-site technologies (e.g., environmental DNA [eDNA]), the majority of conventional methods typically rely on visual observation and counting (Thomsen et al., 2012). The Department of Conservation in New Zealand recommends several sampling methods for koi carp, including passive nets, visual observation, and electrofishing (Grainger et al., 2014). These, and other detection methods commonly used in other parts of the world, are outlined below.

### **1.4.1 Visual observation**

Visual searches for target species in lakes, rivers, and ponds are often carried out in shallow waters in the warmer months. The searcher typically wears polarising sunglasses to reduce glare and increase visibility of the fish (Grainger et al., 2014). This technique requires knowledge of a variety of aquatic taxa, as subtle variations among species can present difficulties for those not proficient in this area. Visual searches are rather challenging with koi carp because their feeding behaviour suspends fine sediments, reducing water clarity and providing a natural cloaking system that decreases their chances of being spotted. This often results in false-negatives (i.e., failure to detect target organism when present), which can lead to inaccurate habitat models and the erroneous use of conservation resources (Tyre et al., 2003).

#### **1.4.2 Passive nets (gill, trammel, fyke)**

Stationary nets are often deployed along the edges of lakes and rivers in the hope of ensnaring particular fish that are suspected to reside there. The size of the mesh determines which fish will be caught and which will pass through the net unaffected (Grainger et al., 2014). One of the issues with passive capture techniques is that mature koi carp tend to become “gear-shy” over time and learn to avoid the nets, leading to biased estimations of population density (Beukema & de Vos, 1974; Clark, Willis, & Berry, 1991). Passive nets also require some degree of human involvement to ensure that they are correctly set up and monitored over time.

#### **1.4.3 Seine nets**

Seine nets are pulled through the water by hand or boat, with both ends of the net slowly closing towards the centre to trap small or juvenile fish inside (Grainger et al., 2014). As with passive nets, koi carp often learn to avoid seine nets if they are used repeatedly, leading to unreliable estimates of fish abundance (Beukema & de Vos, 1974). Debris (e.g., boulders, tree branches, and roots) often needs to be cleared prior to seining in shallow waters to ensure that the net does not get caught or entangled (Grainger et al., 2014), and for these reasons, seining is generally not recommended in swampy or murky areas.

#### **1.4.4 Radio- and acoustic-telemetry (i.e., tagging)**

Using tagging, fish are caught and implanted with radio or acoustic transmitters that enable their movements to be tracked remotely over several months (Daniel et al., 2011). This is often referred to as the ‘Judas’ technique for its ability to

identify carp aggregations, as a single tagged fish can lead researchers to an entire shoal, which can then be removed via point-source control methods. Koi carp have a tendency to aggregate during breeding and across the winter months, and methods such as tagging have been useful in identifying such aggregations (Bajer et al., 2011; Penne & Pierce, 2008). One of the more prominent concerns regarding the use of tagging for the detection of invasive species is that a large quantity of fish must first be captured, released, monitored over an extended period, and then recaptured some time later. This process is not only expensive and time-consuming, it also requires knowledge of recruitment sites prior to conducting a survey, which makes this method less useful for obtaining presence-absence data. Furthermore, estimates of transmitter expulsion from koi carp in the Waikato suggest that as few as 40% of transmitters are retained once implanted (Daniel, Hicks, Ling, & David, 2009).

#### **1.4.5 Electrofishing**

Boat electrofishing typically involves a three-person crew (one driver and two dipnetters) operating 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, Daniel, Ling, Morgan, & Gauthier, 2015a; Hicks, Osborne, & Ling, 2006). This electrical field produces taxis in nearby fish, triggering muscle contractions and causing them to swim involuntarily towards the submerged anodes (Hicks, Jones, de Villiers, & Ling, 2015b). Fish become stunned (narcosis) upon arriving at an anode, often floating belly-up to the surface as a result. They are then quickly retrieved with dip nets before the narcosis subsides (Osborne, 2006). Backpack electrofishing can be carried out in areas not readily

accessible by other means, particularly in shallow waters and those containing fallen trees and other submerged materials (Donkers, Patil, Wisniewski, & Diggle, 2012). Boat electrofishing is commonly used to detect the presence of invasive fish and generate biomass estimates. However, as with other methods, fish must first be caught and counted. An effective fishing field appears to extend 4 m in width in front of the boat and reach depths of 1-2 m below the water's surface (Hicks et al., 2015b). This makes boat electrofishing particularly effective in shallow water, but ill-advised in deeper areas due to its tendency to underestimate species abundance (Banks & Hogg, 2015; Magnuson, Benson, & McLain, 1994). Electrofishing is also relatively expensive compared to other methods. Conservative accounts suggest costs of around \$480 NZD/person-day, not including those associated with travel, equipment, consumables, depreciation, and maintenance (Hicks et al., 2015a).

Some authors have also raised concerns regarding the impact of electrofishing on non-target species. For instance, during any electrofishing survey, fish other than the target species may be exposed to electrical fields. Several studies have documented the injury, and in some cases mortality, of aquatic species during electrofishing expeditions; many of which go undetected unless externally obvious or severe (Snyder, 2003b). Dalbey, McMahon, and Fredenberg (1996) studied the effects of electrofishing on wild rainbow trout (*Oncorhynchus mykiss*) and found that around 40% of trout captured during the study sustained spinal injuries as a result of being exposed to electric shocks. The majority of fish injured (28%) displayed impaired growth and weight loss up to one year later. A study by Culver and Chick (2015) investigated the prevalence of injury

among four native (bluegill, *Lepomis macrochirus*; channel catfish, *Ictalurus punctatus*; freshwater drum, *Aplodinotus grunniens*; and gizzard shad, *Dorosoma cepedianum*) and two invasive (common carp, *Cyprinus carpio*; and silver carp, *Hypophthalmichthys molitrix*) freshwater fish species captured using electrofishing in two North American rivers. Although no injuries were detected among common carp, bluegill, freshwater drum, and gizzard shad, 27% of channel catfish and 50% of silver carp displayed vertebral fractures and associated haemorrhages during necropsy examinations, suggesting that certain species may be more susceptible to injury by electrofishing than others. In another experiment, Henry, Grizzle, and Maceina (2003) studied the effects of electroshock on four fish species (large-mouth bass, *Micropterus salmoides*; bluegill, *Lepomis macrochirus*; channel catfish, *Ictalurus punctatus*; and Nile tilapia, *Oreochromis niloticus*) during larvae and juvenile stages of development. Across all four species, fish less than 100 days old were particularly susceptible to death when exposed to electrical currents similar to those used in electrofishing surveys. Age (days) and length of all fish were negatively correlated with mortality. No information regarding the effects of juvenile fish injury on later adult development was provided, so inferences about harmful effects other than mortality (e.g., impaired growth) cannot be made.

While there is little doubt that electrofishing can be damaging to exposed fish, comparable rates of injury have been reported using other capture methods. For instance, de Villiers (2013) found that gill netting, fyke netting, seine netting, and electrofishing, produced similar rates of stress, injury, and mortality in three native freshwater fishes (shortfin eel, *Anguilla australis*; grey mullet, *Mugil*

*cephalus*; and common smelt, *Retropinna retropinna*) in the Waikato region, although haemorrhaging was more prevalent after electrofishing for eels and grey mullets. Fish injuries can also result from careless handling during capture and transport (Snyder, 2003a).

#### **1.4.6 Environmental DNA**

In order to eliminate the risk of damage to non-target species and associated habitats, less invasive detection procedures should be considered. Environmental DNA is one such alternative to conventional fish detection methods. Using this technique, researchers isolate DNA fragments (e.g., sloughed skin cells) obtained from environmental samples and use these as the detection target, rather than the species itself (Lodge et al., 2012; Takahara, Minamoto, & Doi, 2013). This is done by extracting and amplifying small sections of genes using polymerase chain reactions (PCR) that contain species-specific primers and synthetic nucleotides. If these complimentary portions of DNA match the target sequence, they bind to the gene template and produce amplicons that can be visualised using electrophoresis on an agarose gel, or detected by fluorescent luminance after DNA synthesis (Banks & Hogg, 2015; Dejean et al., 2011; Wood et al., 2013). Quantitative PCR (qPCR) allows for the quantification of eDNA sample concentrations and can be performed in real-time with the addition of species-specific probes after the DNA has replicated. This replication only occurs if the primer precisely corresponds to the target DNA, meaning that the DNA of other organisms is ignored. Because of its ability to detect isolated fragments of genetic material in the water column, this method can identify the presence of organisms not otherwise detectable by traditional survey methods. Although a large amount of data confirming the utility

of this method exists, eDNA is not currently used to monitor koi carp in New Zealand (Banks & Hogg, 2015).

Environmental DNA does not rely on observing or capturing fish and has been used to detect both marine and freshwater fish species. One of the benefits of using this method is that it has little to no impact on the habitats from which the water is sampled. A number of studies have demonstrated its efficacy in detecting a wide-range of fish. For example, Kelly, Port, Yamahara, and Crowder (2014) used qPCR assays to assess biodiversity in a 4.5-million-litre marine mesocosm and found that it was able to accurately detect bony fishes such as sardines and tuna. Turner, Miller, Coyne, and Corush (2014) used both PCR and qPCR assays to detect the presence of Asian bigheaded carp (*Hypophthalmichthys spp.*) in an experimental pond. They found that qPCR assays produced a detection probability of 94.8%.

Several studies have also sought to detect eDNA in running water. Goldberg, Pilliod, Arkle, and Waits (2011) confirmed the efficacy of eDNA detection methods in swift-moving water by extracting the DNA of two elusive freshwater amphibian species (Rocky Mountain tailed frogs, *Ascaphus montanus*, and Idaho giant salamanders, *Dicamptodon aterrimus*) using PCR assays at known recruitment sites. In another study, Thomsen et al. (2012) tested water samples from 98 natural ponds, streams, and lakes around Europe known to contain endangered species from different taxonomic groups (i.e., fish, amphibians, insects, crustaceans, mammals). The authors used qPCR assays to extract and detect DNA from target species, comparing the detection rates across both

running and stagnant water systems. Detection probabilities of 100% were found in ponds and 54% in running water for the European weather loach fish (*Misgurnus fossilis*).

A further experiment by Thomsen et al. (2012) investigated the persistence of eDNA in a controlled freshwater mesocosm and found that the eDNA of amphibian larvae were detectable up to two weeks after removal. Dejean et al. (2011) found similar rates of eDNA persistence for Siberian sturgeon (*Acipenser baerii*; 14 days) and bullfrog tadpoles (*Lithobates catesbeianus*; 25 days). In lotic systems, however, DNA fragments appear to dissipate considerably faster. Balasingham, Walter, and Heath (2017) released Atlantic salmon (*Salmo salar*) water into a flowing river and were able to detect DNA at the source site up to 11.5 hours later. Both Balasingham et al. (2017) and Shogren et al. (2017) found significantly lower levels of eDNA only slightly downstream from the original source, likely due to the effects of substrate settling, dilution, and physical degradation during suspension and transport. This rapid decline of genetic material suggests that the discovery of extracellular DNA fragments in the environment is indicative of recent species presence.

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 (Jerde, Mahon, Chadderton, & Lodge, 2011). That is, estimates of biomass density may be inferred from the prevalence of eDNA fragments. This could be true in stagnant water systems, but flow rates in lotic areas affect dilution levels so conclusions based on signal strength alone are not advised.

The utility of eDNA-based detection methods over other detection methods can be determined as a function of its ability to provide improved sensitivity at a similar or reduced cost. Sigsgaard, Carl, Moller, and Thomsen (2015) compared eDNA with conventional methods for the detection of the European weather loach fish (*Misgurnus fossilis*) and 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). Smart et al. (2016) compared high- and low-cost eDNA methods with trapping for the detection of an aquatic newt species (*Lissotriton v. vulgaris*), with the high-cost method also screening for taxonomically-similar species to reduce false error rates. Low-cost eDNA sampling appeared to be more cost-efficient than trapping but was prone to errors due to less stringent qPCR thresholds. The authors concluded that conventional trapping methods, although less sensitive, may be more cost-efficient for surveys with larger budgets.

While eDNA appears to offer increased sensitivity over conventional methods, some authors have cautioned that this extreme sensitivity may lead to the increased likelihood of false positives through DNA contamination. Piscivorous birds and mammals in the wild, for example, have been known to scatter prey DNA far from its point of origin through predation or secondary predation (Symondson, 2002). Secondary predation refers to the consumption of a predator by a secondary predator, in which the first has consumed the target prey (e.g., a bird eats a fish and is then devoured by a cat, leaving remnants of the fish in the cat's stomach). The scats of these animals can also contaminate fluvial networks and be transported to other environments through the water, meaning that any DNA

confirmed present may originate from sources other than live organisms. In their large mesocosm experiment, Kelly et al. (2014) found that over a quarter (25.5%) of DNA found were exogenous DNA sequences attributed to species not present in the tank. The majority (62%) of this DNA came from humans, and the remainder was comprised of cow, pig, chicken, turkey, and anchovy DNA, which likely came from fish feed. It is fairly easy to surmise that a pig is not present in a tank of water, even if its DNA is discovered there. However, this becomes less obvious when other likely candidates are detected (i.e., other species of fish). Stoeckle, Soboleva, and Charlop-Powers (2017), for instance, collected water from estuaries and found that 10% of water samples contained contaminant DNA from rare or absent fish, likely originating from nearby sewage systems. This is particularly concerning given its potential to generate false-positive indications, which may lead to misguided assumptions of species abundance.

An additional consideration involves the presence of hybrid species. In order to detect the presence of a particular species using eDNA, the species' genetic code must first be sequenced and developed into a primer. This is a relatively costly and time-intensive process that is predicated on expertise in molecular genetics (Stoeckle et al., 2017; Wood et al., 2013). Also, if the DNA of a particular species has not been sequenced, that species cannot be detected (Venter & Bezuidenhout, 2016). In New Zealand, koi carp and goldfish have been known to interbreed and produce hybrid offspring (Collier & Grainger, 2015). If eDNA technologies are to be used to detect either of these species, probes and primers that are not sufficiently specific can result in high false positive and false negative detection rates (i.e., the presence of hybrid species may lead to the

conclusion that one or both target species are present, resulting in overestimations of species distribution; Wilcox et al., 2013). Because of this, more research is needed to evaluate false error rates and the implications that this may have for conservation (Banks & Hogg, 2015; Deiner, Fronhofer, Machler, Walser, & Altermatt, 2016).

#### **1.4.7 Conclusion**

Conventional methods employed in the detection of native and invasive freshwater fish species leave much to be desired; they are expensive, time-consuming, invasive, and prone to error. Tyre et al. (2003), for instance, found that as many as six repeated visits are often needed to correct false-negative detection rates when using conventional detection methods (e.g., netting, tagging, visual observation) to obtain presence-absence data during ecological surveys. This not only significantly increases the cost of surveys, it is also time-consuming and inefficient from a conservation perspective. Researchers have sought to correct these issues by developing procedures, such as eDNA, that minimise habitat disturbance. While eDNA does appear to be more sensitive, less costly, and relatively non-invasive, it does not address the problem of false error rates. What is needed going forward is a method for identifying the presence of numerous fish species that is cost-efficient, sufficiently (but not overly) sensitive, and relatively easy to implement, with little to no impact on the organisms under study.

## 1.5 Could dogs be the answer?

### 1.5.1 Dogs' biological preparedness for scent detection work

Domestic dogs (*Canis familiaris*) have an incredibly sensitive sense of smell. Their ability to detect volatile organic compounds (VOCs) has been demonstrated in a number of experimental and applied studies. One study using n-amyl acetate as the target scent found that dogs were able to detect lower limit concentrations of approximately one part per trillion; the equivalent of a single drop of liquid in 20 Olympic-size swimming pools (Walker et al., 2006). This extreme olfactory sensitivity means that dogs reliably outperform even the most advanced biotechnologies available to date (Angle, Waggoner, Ferrando, Haney, & Passler, 2016).

Olfaction typically involves two components: the detector (e.g., the nose), and the analyser (e.g., the brain; Galibert, Azzouzi, Quignon, & Chaudieu, 2016). In dogs, both of these components are well-equipped to detect odours, particularly when compared to humans and several other mammals. One study, for instance, found that dogs' olfactory brain parts (e.g., olfactory bulb, stria, and tract), are much larger and make up a greater proportion of the total brain volume than those of humans (i.e., 1.95% of total brain volume in dogs compared to 0.03% in humans; Kavoi & Jameela, 2011). This is not surprising given that domestic dogs evolved from canid ancestors who relied primarily on olfaction to track and capture prey species, and also to communicate with conspecifics. Those who were better prepared, biologically, were able to detect subtler olfactory cues and enjoyed a distinct advantage over competing predators (Nowak, 2005).

### **1.5.2 Applied scent detection research with dogs**

Dogs' olfactory capabilities have been used for a variety of scent detection tasks (Johnen, Heuwieser, & Fischer-Tenhagen, 2013). For instance, dogs have been used successfully in the detection of narcotics (Jeziński et al., 2014), various cancers (Guerrero-Flores et al., 2017; McCulloch et al., 2006; Sonoda et al., 2011) and other diseases (Alasaad et al., 2012; Koivusalo et al., 2017), land mines (Lazarowski & Dorman, 2014), human remains (Alexander, Hodges, Bytheway, & Aitkenhead-Peterson, 2015; Oesterhelweg et al., 2008; Riezzo et al., 2014), and even cows in oestrus (Fischer-Tenhagen, Wetterholm, Tenhagen, & Heuwieser, 2011), to name but a few of many applications.

#### **1.5.2.1 Cryptic species detection**

Dogs have proven to be valuable tools in environmental conservation work for the detection of both plant and animal species. In the detection of plants, for instance, Sargisson, Popay, McLean, and Crocker (2010) found that dogs were able to distinguish between 19 plant species, and were capable of detecting pest plants in their natural environments. Goodwin, Engel, and Weaver (2010) found that dogs outperformed human surveyors in the detection of rare spotted knapweed (*Centaurea stoebe*), demonstrating higher detection accuracy for small, medium, and large plants, and indicating the weeds' presence at a greater distance from the target.

Dogs are also recognised for their ability to identify the presence of animals where traditional methods, such as visual searches, are limited. For example, insects, while not always readily visible to humans, can produce a number of

undesirable effects from the disruption of terrestrial ecosystems (e.g., Asian longhorn beetle, *Anoplophora glabripennis*; red imported fire ants, *Solenopsis invicta*), to the injuring of humans and other animals (e.g., bed bugs, *Cimex lectularius* L.), and the attrition and collapse of entire buildings (e.g., termites). Several studies have demonstrated dogs' ability to accurately detect a variety of cryptic insect species. Hoyer-Tomiczek, Sauseng, and Hoch (2016) trained 14 dogs to detect the scents of Asian longhorn beetles by concealing wood shavings and frass around the base of trees and at 1.8 m high tree crevices. Lin et al. (2011) successfully trained three dogs to locate red imported fire ants and associated nests in the field. Pfiester, Koehler, and Pereira (2008) investigated the ability of dogs to detect bed bugs in hotel rooms and found that dogs were able to locate live bed bugs with 98% accuracy and discriminate live bed bugs and eggs from dead bed bugs, scats, and shed skins. Brooks, Oi, and Koehler (2003) successfully trained six dogs to detect five species of termite, and discriminate termites from termite-damaged wood and other insects, including ants and cockroaches.

Dogs have also been used to identify larger invasive terrestrial species for eradication. Gsell, Innes, de Monchy, and Brunton (2010), for instance, found that trained dogs were able to locate 87% of Norway rats (*Rattus norvegicus*) and 80% of mice (*Mus musculus*) that were released into a 63-ha fenced forest sanctuary, with few false-positive detections. Savidge, Stanford, Reed, Haddock, and Adams (2011) used dogs to detect invasive brown tree snakes (*Boiga irregularis*) in a tropical forest and found them to be superior to human visual searching.

In addition to the identification of invasive species, dogs have proven capable of detecting the scents of other terrestrial animals for the purposes of conservation. For example, dogs outperformed humans in the search for desert tortoises (*Gopherus agassizii*) and associated burrows with overall detection accuracies exceeding 90% (Cablak & Heaton, 2006). Chambers, Vojta, Mering, and Davenport (2015) trained dogs to detect guano and elevated bat roosts and indicate their location to within a 30 m radius above 70% accuracy. Browne, Stafford, and Fordham (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 across trials. Long, Donovan, Mackay, Zielinski, and Buzas (2007) found that detection dogs were effective at locating the scat of three forest carnivores (black bears, *Ursus americanus*; fishers, *Martes pennanti*; and bobcats, *Lynx rufus*) while ignoring the scat of other species in a real-world field survey.

#### **1.5.2.2 Detection of underwater targets**

There is a large body of research demonstrating the efficacy of scent detection dogs for terrestrial searches, but few studies have been published regarding their ability to locate underwater targets; the data that do exist detail the use of dogs for human cadaver detection. According to Koenig (2000), dogs have been used in the search for submerged corpses since the mid-1970s. During this process, trained dogs typically stand at the bow of the boat and sniff the water's surface as the vessel moves from bank to bank in a predetermined grid pattern. Dogs are also sometimes deployed along the shore line or allowed to swim in slow-moving waters to locate target scents. Once the dog detects the scent of a cadaver, it

alerts the handler with a pre-trained alert response and the area is investigated more thoroughly to discover the source of the scent.

In a recent review of the literature, Osterkamp (2011) admits that information regarding the nature of scents and scent transport processes underwater is lacking, but reasonable hypotheses might be generated from knowledge regarding the behaviour of submerged gases, liquids, and solids, and also how VOCs spread in the terrestrial environment. Gases, for example, typically travel up through the water to the surface. Dogs, therefore, do not smell items *through* the water; rather, certain organic compounds excreted from submerged organisms rise through the water and evaporate into the air above the surface where they are available to be detected by the trained dog. Given dogs' ability to detect submerged cadavers, it is possible that they may also be able to detect other organisms (i.e., freshwater fish) below the surface of the water.

### **1.5.3 Limitations of applied and experimental scent detection tasks**

The ability of dogs to detect novel scents, such as freshwater fish, must be examined in a robust manner by using scientifically sound procedures that leave little to be contested. In order to do this, the limitations of previous scent detection research must be examined and addressed.

#### **1.5.3.1 Line-up arrangements**

In experimental studies, scent samples are often placed in a line-up arrangement. During this type of experiment, the handler takes the dog to each sample, one at a time, and records the dog's responses. However, even under ideal conditions the handler may misread the dog's behaviour, resulting in misses (i.e., incorrectly

indicating the absence of the target scent) or false alarms (i.e., incorrectly indicating the presence of the target scent; Rebmann, David, & Sorg, 2000). During line-up work, there is also the potential for human cueing, even under double-blind conditions. In a four-sample line-up, for instance, if the dog indicates negative on the first three samples, the handler may be led to believe that the final sample must be positive. This could result in the handler inadvertently cueing the dog to respond positively to the sample (e.g., they may force the dog to spend longer evaluating the sample or emit micro-expressions that have historically been associated with positive samples).

#### **1.5.3.2 Limitations associated with field work**

Scent detection tasks in the field typically involve a dog and handler duo. The handler takes the dog to sites of interest where the dog emits either a pre-trained alert response to indicate the presence of the target scent, or no response to indicate the absence of the target. In applied research, dogs and handlers typically visit multiple locations to evaluate the presence/absence of the scent target. This process is time-consuming, particularly if sites are sparsely located, thereby increasing the time and costs associated with trips to multiple areas. The issue is further compounded by the fact that multiple dogs (and, therefore, handlers) are often needed to evaluate the same sample in order to reduce false errors. The ability to perform site visits is also affected by weather conditions, such as temperature, wind speed, and precipitation (Rebmann et al., 2000), which have also been known to affect scent detection accuracy. Some authors, for example, have found that dogs are less likely to detect target scents at lower temperatures

(Chambers et al., 2015; Sargisson et al., 2010), and under strong wind and less humid conditions (Savidge et al., 2011).

### **1.5.3.3 Handler subjectivity and cueing**

A discussion of the limitations of tasks involving human-animal interactions, and consequently, detection dog performance, would not be complete without reference to human bias. In the early 1900s, there were reports of a horse named Clever Hans who was able to perform mathematical equations and answer general knowledge questions by tapping his hooves to indicate the numbers or letters he wished to communicate (Samhita & Gross, 2013). Hans' understanding of mathematics and the general affairs of the time was, of course, demonstrably false; he was responding to subtle micro-expressions on the face of the questioner and gestures of others in the crowd, not the actual content of their speech. The example of Clever Hans highlights the potential for unintentional cueing and bias in human-animal interactions. Indeed, a "Clever Hans effect" has been reported in numerous studies of canine olfaction. Szeteci, Miklosi, Topal, and Csanyi (2003), for example, found that the presence of human cueing was sufficient to override visual and olfactory cues associated with hidden food. Lit, Schweitzer, and Oberbauer (2011) demonstrated that handler beliefs of the location of the target scent significantly affected dogs' performance, even under double-blind conditions. That is, when handlers were falsely alerted to the presence of the target scent, dogs became significantly more likely to indicate positive even in the absence of the scent. Zubedat et al. (2014) found that increased handler stress was associated with superior dog performance in a double-blind test of explosive detection. The authors hypothesised that higher stress levels impaired handler

attention, leading to less control over dogs' leashes, and consequently, reduced cueing from handlers. It has also been reported that dogs' accuracy during scent detection tasks decreases when handlers are changed (Nolan & Gravitte, 1977, as cited in Zubedat et al., 2014), and that certain idiosyncrasies among owners, such as their gender and personality, influence dogs' behaviour and subsequent success in operational tasks (Kotrschal, Schoberl, Bauer, Thibeaut, & Wedl, 2009). Therefore, in order to test the true ability of dogs during scent detection tasks, human influence must be removed.

#### **1.5.4 Towards an improved scent detection procedure**

With the ultimate purpose of conservation in mind, any modifications to traditional scent detection procedures in experimental settings must also be readily applied under operational conditions. In signal detection research, one of two methods is typically used: the yes/no procedure and the go/no-go procedure (Moret-Tatay & Perea, 2011). In a yes/no task, subjects are presented with a stimulus and are required to make a "yes" or "no" response to indicate whether that stimulus matches the target stimulus. Correct responses (i.e., hits and correct rejections) are reinforced, and incorrect responses (i.e., false alarms and misses) are either punished or receive no consequence. In a go/no-go task, subjects perform the "yes" response if the stimulus matches the target, and refrain from responding if it does not. During this procedure, only hits (i.e., correctly indicating the presence of the target scent) result in reinforcement, while all other responses have no programmed consequences.

Laboratory research typically uses the yes/no method, and field research often uses the go/no-go method. The current study is experimental in nature, but for an experimental procedure to be successful outside of the laboratory it must be able to accommodate real-world sample availability. In the detection of rare or hidden species, it is often difficult to ascertain the true nature of a sample, and while using the yes/no method, accidental reinforcement of false positive samples (i.e., incorrectly indicating the presence of the target scent) can have serious negative consequences for overall detection accuracy. On the other hand, intermittent reinforcement of hits is unlikely to result in such adverse effects. The go/no-go procedure is advantageous in this regard because it does not require contingencies to be arranged for negative samples and demands only occasional reinforcement of positive samples to maintain responding. This procedure more closely resembles what would be ideally used in an applied scenario.

While most applied research involves a handler bringing the dog to the sample site, a number of limitations associated with these methods have been explained above. In order to avoid these issues, samples could be brought to the dog who then works independently in the absence of a handler. This could be achieved through the use of an automated apparatus that presents scent samples to the dogs and evaluates their level of accuracy relative to each sample.

#### **1.5.5 Purpose of the present study**

All animals, living and dead, generate waste (e.g., sloughed skin cells, excrement) that is released into the environment where it persists for a period of time before disintegrating (Kelly et al., 2014). This waste is made up of certain organic

compounds, some of which contain volatile elements that disperse into the air. It is possible that organisms living under the water generate waste that contain unique VOC signatures (similar to that of terrestrial animals) that rise through the water and can be detected at the surface by trained dogs. While this is a plausible hypothesis, no published research so far has examined the ability of dogs to do this.

The aim of this study was to investigate the utility of dogs for the detection of freshwater fish. This study was designed with three research questions in mind:

1. Can dogs detect the presence of fish using water samples that have contained those fish?
2. If so, are dogs able to do detect these fish under similar biomass densities as those observed in the natural environment?
3. Are dogs able to distinguish between multiple species of fish (i.e., those distantly- or closely-related to the target fish)?

# Chapter Two

## Experiment 1: Acquisition of the target scent

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### 2.1 Introduction

Koi carp represent a significant threat to freshwater ecosystems worldwide (Cahn, 1929; Huser et al., 2016; King & Hunt, 1967; Kloskowski, 2011). To alleviate the negative impact that these fish have on the environment, they must first be detected using the appropriate methods. However, current methods employed in the detection of these fish are not sufficient (Beukema & de Vos, 1974; Daniel et al., 2009; de Villiers, 2013; Snyder, 2003a; Stoeckle et al., 2017). In order to address the limitations associated with these procedures, new methods of enquiry must be tested. Dogs are known for their excellent olfactory capabilities (Walker et al., 2006), but are not currently used in the detection of freshwater fish. The aim of this experiment was to determine whether dogs can detect the presence of fish from water samples, and therefore demonstrate a novel application of dogs for the detection of underwater organisms.

### 2.2 Method

#### 2.2.1 Ethics

Approval for all experiments in this thesis was obtained from the University of Waikato Animal Ethics Committee (AEC; Protocol #1013). Dog owners were given information about the study, including the purpose and methodology, and had the opportunity to ask questions before consenting to have their dogs participate. A copy of the consent form can be found in Appendix B.

Table 1.  
*Details of dogs recruited for participation in this research.*

Name	Sex	Age (years)	Breed	Passed training?	Experiments participated in			
					1	2	3	4
Luna	F	3	Border collie X (cross)	Yes	Yes	Yes	Yes	Yes
Ruby	F	5	Labrador retriever X border collie	Yes	Yes	Yes	Yes	Yes
Mica	F	5	Blue heeler X heading dog	Yes	Yes	Yes	Yes	Yes
Louis	M	1	Wire-haired dachshund X shih tzu	Yes	Yes	Yes	No	No
Vincent	M	9	Dachshund	Yes	Yes	Yes	No	No
Ronnie	F	6	Pug X Jack Russell	No	No	No	No	No
Nash	M	8	Unknown	No	No	No	No	No
Max	M	0.8	Rhodesian ridgeback	No	No	No	No	No
Cocoa	F	10	Labrador retriever	No	No	No	No	No
Kikko	M	0.6	Unknown	No	No	No	No	No
Boy	M	0.3	Fox terrier	No	No	No	No	No
Kimchi	F	11	Papillion X	No	No	No	No	No
Hudson	M	4	Yorkshire terrier X poodle	No	No	No	No	No

All fish were held under an existing Standard Operating Procedure (SOP) and met AEC standards.

## **2.2.2 Subjects**

### **2.2.2.1 Dogs**

Thirteen pet dogs were recruited from owners in the Waikato region, New Zealand, through flyers, online social media, and word of mouth. The dogs varied in age, breed, sex, and previous level of training (Table 1). Dogs were not accepted on the basis of physical characteristics or breed alone, as current evidence suggests that there is more behavioural variation within breeds than among them (Hall, Glenn, Smith, & Wynne, 2015). Instead, dogs were selected based on answers to an initial screening form (see Appendix A) that was administered to owners prior to their dogs participating in the study. The first stage of selection (i.e., the screening form) was designed to assess factors such as motivation for food, friendliness towards other dogs and people, and behaviours associated with neophobia and separation anxiety. The second stage of selection was designed to test the performance of each dog in early training sessions. Of the 13 dogs that were initially recruited, eight were excluded from the study for reasons such as being disinterested in the food provided, showing little motivation to work, being reliant on prompts and lures to such a degree that they would not work in the absence of prompting (even when significant time was taken to fade out these prompts), and demonstrating anxious behaviour (e.g., trembling, vocalisations, and inappropriate elimination). Consequently, of the 13 dogs that were recruited

over a period of five months, five of these dogs passed the early training stage and went on to participate in subsequent experiments.

#### **2.2.2.2 Fish**

Four wild koi carp sourced from local waterways were placed in freshwater tanks, located at the Aquatic Research Facility at the University of Waikato, New Zealand.

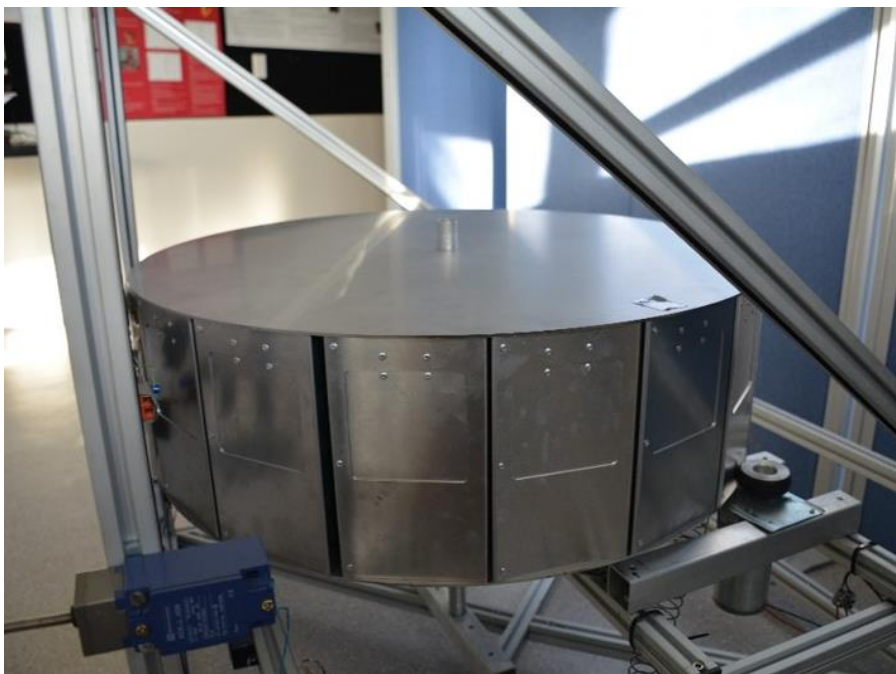
#### **2.2.3 Study location and apparatus**

Experiments were conducted twice weekly (i.e., Thursday and Friday) during either morning (8:30 am – 12 pm) or afternoon (1 pm – 4:30 pm) sessions at the canine research facility located at the University of Waikato, New Zealand. The facility comprised several rooms: an office, a kitchen, an experimental room, and a control room where dogs were kept between experiments.

The experimental room was approximately 3.2 m x 4.3 m and contained the automated apparatus (Figures 1-3). The apparatus was a custom-built 1 m<sup>3</sup> circular device with an aluminium frame containing 17 removable segments where samples were placed. The apparatus rotated to present individual scent samples to dogs, one sample at a time, through a 10 cm port in the front panel. Attached to the front panel on the right side of the apparatus was an omnidirectional limit switch that could be triggered to rotate the apparatus clockwise to present the next sample. During experimental sessions, a metal lid was placed on the top of the apparatus to ensure that each segment was completely sealed. Each of the 17 segments contained a flap in the front into which dogs could insert their nose, via the port, to gain access to a scent sample.



*Figure 1.* Top-view of the apparatus with the lid removed displaying each of the 17 segments. Sample jars containing water were placed in each of the 17 segments.



*Figure 2.* Side-view of the apparatus with the lid attached. The blue limit switch is fixed to the front panel (bottom left).



*Figure 3.* Luna (left) and Vincent (right) sitting in front of the port of the apparatus (i.e., the grey circle next to Luna's head). Vincent is standing on a wooden platform to enable him to reach the apparatus port.



*Figure 4.* Mica evaluating a scent sample. The automated feeder (bottom left) was moved forward approximately 1 m in order to capture the apparatus and the feeder in a single photograph.

Only the front panel of the apparatus was accessible to dogs, meaning that only one segment of the apparatus (and thus one sample) was presented at a time. When dogs inserted their nose into the port of the apparatus and pushed open the segment flap, an infrared beam was broken, which resulted in a 'beeping' sound and the automatic recording of the duration of the sniffing response. A remote automated feeder (PetSafe Treat & Train™ Remote Reward Dog Trainer) was situated on the floor approximately 2 m in front of the apparatus. This was controlled by the computer, which delivered one piece of dry Pedigree® kibble when a reinforcer was triggered. Data were collected electronically and recorded in real-time on a computer (Dell™ Optiplex 780 running Windows Vista™) in the adjacent control room using a custom-designed software application. The experimental room also featured two video cameras (Logitech® 2 MP HD Webcam C600) with built-in microphones to provide complete real-time video coverage of the room, and to allow for dogs to be monitored from the control room without also requiring that the experimenter be present in the experimental room with the dog.

#### **2.2.4 Aquatic centre**

Three aquaria (one containing no fish, and two containing koi carp) were used in this experiment to prepare water samples for presentation to the dogs. Each tank was identical in terms of material composition and size (material: fibreglass; dimensions: 59 cm high x 72 cm long x 81 cm wide; total water volume: 230.4 L). Tanks received fresh dechlorinated water through a hose situated above the tank, and oxygen was supplied to each tank through a submerged plastic tube. Tank 1 contained only freshwater (no fish) and was used as the control water tank during

this experiment. Tanks 2 and 3 contained two koi carp each (range: 1.72 – 2.02 kg/tank). Fish were fed standard fish pellets each Monday. On Wednesdays and Thursdays, tanks containing fish were cleaned (i.e., scrubbed with coarse Scotch-Brite™ cleaning pads), rinsed with water, drained, and refilled to approximately half of their full capacity (115.2 L). After cleaning and refilling each tank, the water flow was stopped for 24 hours prior to sample collection. A sampling volume of 115.2 L was selected as this enabled a balance of both fish welfare (i.e., ensuring that the fish had enough water in which to swim around freely), and sample potency, as previous research has suggested that initial target odours should be sufficiently salient to facilitate acquisition of the target scent (Jeziarski et al., 2014).

### **2.2.5 Sample collection**

Water samples were collected on experimental days from both fish and no-fish tanks. In order to minimise fish disturbance, samples were collected from Tanks 1 (no fish) and 2 (fish) on Thursday, and from Tanks 1 (no fish) and 3 (fish) on Friday. Water was collected using 250 mL glass beakers which were then emptied into 2 L glass bottles with lids and transported to the dog facility once filled. Separate glass bottles and beakers were allocated to each tank type and no-fish (i.e., negative) samples were always collected first to avoid contamination. Disposable latex gloves were used and changed frequently between all stages of the collection process. Standard operating procedures that were followed during sample collection can be found in Appendix C.

Fish and no-fish sample bottles were stored separately in a refrigerator at approximately 4°C during the morning until 12 pm when sample jars (i.e., those used during the experiment) were refreshed in preparation for afternoon sessions. This allowed for the samples to be preserved, but also for the water to revert back to room temperature before the afternoon sessions began at 1 pm.

### **2.2.6 Sample preparation**

Water samples were prepared at the dog facility on the morning of collection prior to conducting experiments. Negative samples were always prepared first using a 100 mL plastic syringe to transfer 100 mL of water from no-fish bottles into glass cosmetic jars (60 mm high x 89 mm wide). These jars were labelled with a single rectangular adhesive label placed horizontally across the front of the jar. Positive samples were prepared similarly using a separate 100 mL plastic syringe by transferring 100 mL of fish water into glass jars. Positive sample jars were marked in the same manner as negative jars but the label was placed vertically, rather than horizontally. In the early stages of the experiment, smaller glass sample jars filled with approximately 45 mL of liquid were used. These smaller jars were replaced with the larger cosmetic jars, described above, due to the greater surface area of the liquid inside the cosmetic jars so that more VOCs would be available in the headspace to facilitate detection of the target odour. Disposable gloves were used and changed between the handling of each sample type. Standard operating procedures that were followed during sample preparation can be found in Appendix C.

### **2.2.7 Storage, cleaning, and contamination**

All collection beakers and bottles were cleaned each day after use with hot soapy water, rinsed thoroughly, and dried with disposable paper towels to remove any bacterial growth. At the end of each experiment day, used sample jars were submerged in a concentrated nitric acid ( $\text{HNO}_3$ ) bath for at least 24 hours, and then rinsed thoroughly with distilled water and dried in a Contherm Thermotech 2000 oven to remove any residual odours. At the end of each week, the apparatus was cleaned thoroughly with a 70% isopropanol solution and disposable paper towels, and each segment was washed in hot soapy water, rinsed, and left out to dry.

### **2.2.8 Training**

Dogs were first trained to approach and consume kibble from the feeder, which was operated by the experimenter via remote control. After dogs began to approach the feeder within 3 s of food delivery reliably, their behaviour was shaped to approach the apparatus and place their nose in the sample port using the method of successive approximations. For smaller dogs, a wooden platform was placed in front of the apparatus to enable them to reach the sample port. During this phase, all segments contained positive samples and reinforcement was delivered by the experimenter using the remote feeder each time the infrared beam was broken. Dogs received auditory feedback in the form of a “beep” for as long as the infrared beam remained broken. Once dogs were placing their noses in the sample port reliably, the software programme connected to the apparatus was set to release food only after a response duration (i.e., infrared beam break by a dog’s nose being held in the sample port) of 500 ms. The response duration

requirement was gradually increased in increments of 250 ms until a response duration of 1.5 s was reached. Once dogs were responding for >1.5 s reliably, the experimenter then turned the apparatus off and shaped the behaviour of each dog (i.e., using the remote feeder to deliver food when appropriate) to activate the limit switch on the side of the apparatus. The topography of this behaviour was not important so long as the limit switch was activated; consequently, dogs learned to activate the limit switch using their nose, head, chest, or paws. Negative samples were then added into the apparatus so that every second segment contained a negative sample (i.e., eight negative vs. nine positive).

After the addition of negative samples, the apparatus was turned on and the experimenter stood to the right of the apparatus and prompted dogs to activate the limit switch during negative trials. Prompts were faded out progressively as dogs began to operate the apparatus independently. At all stages of training, the experimenter avoided eye contact with the dogs and minimised interactions to ensure that the dog did not become reliant on human-related cues, and was attending to the task rather than the experimenter. Occasionally, gestural prompts and luring were also used to facilitate the training process, but these were faded out over the course of training. Once the dogs were operating the apparatus independently, the experimenter began slowly fading himself out of the room over subsequent sessions until the dogs were performing sessions in the absence of the experimenter with the door closed. At this point, the response duration was increased progressively (in increments of 500 ms), from 1.5 s to between 3.5 – 4.5 s depending on the performance of each dog during positive and negative trials. For example, if dogs held their nose in the port for longer than

the pre-specified duration reliably during both types of trials, the response duration was increased to encourage them to discriminate between sample types.

During positive trials, two outcomes were possible: hits or misses. Hits were achieved when dogs broke the infrared beam by placing their nose in the port of the apparatus and holding it there for longer than the pre-specified response duration (e.g., 3.5 s). This activated the remote food dispenser which immediately released a piece of dog kibble, and the apparatus then rotated around to the next segment to begin a new trial. Misses were observed when dogs broke the infrared beam but held their nose in the port for less than the pre-specified response duration, and then activated the limit switch on the side of the apparatus, thereby skipping that trial and receiving no reinforcement.

During negative trials, two slightly different outcomes were possible: correct rejections or false alarms. Correct rejections were achieved when dogs broke the infrared beam for longer than 500 ms but not longer than the pre-specified response duration. Once dogs had evaluated a sample, they 'correctly rejected' it by activating the limit switch, which rotated the apparatus around to the next sample. The limit switch remained inactive until dogs had exceeded a period of 500 ms with their nose inside the port to ensure that they were spending sufficient time evaluating each sample and not capriciously skipping trials. As this was a go/no-go procedure, no reinforcer was provided for correct rejections of negative samples, rather a new trial was presented with an additional chance of obtaining a food reinforcer if that trial contained a positive sample. False alarms were observed when dogs broke the infrared beam for longer than the pre-

specified response duration, thereby falsely indicating that the sample was positive. Exceeding the response duration on negative trials was considered incorrect and had no programmed consequences; dogs were simply required to activate the limit switch to access the next trial.

### **2.2.9 Experimental procedure**

Once dogs were operating the apparatus independently, sample arrangements were randomised between sessions to prevent sequence learning effects. The lid and base of the apparatus were cleaned with disposable paper towels soaked in a 70% isopropanol solution at the end of each session before rearrangement of the samples. Initially, separate segments were not allocated for each sample type. It soon became apparent, based on the dogs' performances, that negative samples were becoming contaminated with the target scent when samples were rearranged between sessions. To rectify this, segments were cleaned (i.e., with the isopropanol solution described above) between sessions. However, this was found to be inefficient and unnecessary and after further discussion it was decided that the allocation of separate segments for each sample type was sufficient. Positive segments were then marked with a small adhesive label running vertically along the top of the segment to ensure that segment/sample combinations were never mixed. In order to encourage discrimination, and also reduce satiation and allow for more sessions to be completed, sample proportions were adjusted so that each full rotation of the apparatus contained seven positive and 10 negative samples. One session comprised two full rotations of the apparatus (i.e., 34 trials) and lasted approximately 5 min. This was done initially to enable the dogs to have more exposure to samples to facilitate learning, and also to provide a larger data

set from which to derive the average accuracy rates. However, it was also useful in identifying possible contamination of negative samples (i.e., when multiple dogs indicated positive on a negative trial during both rounds, it was likely that the samples may have inadvertently become contaminated with the target scent). Once the apparatus had presented all 17 segments, it continued to present segments in the same order until two full rotations were completed. It is unlikely that the dogs received any cues to signal the end of the first rotation and the beginning of the second.

A termination criterion of four consecutive sessions above 80% accuracy across both positive and negative samples was set, and dogs were deemed to have successfully completed Experiment 1 once they achieved this.

#### **2.2.10 Data analysis**

Data were analysed using descriptive statistics to derive the average accuracy rates for each sample type (i.e., hits and correct rejections), standard deviation, and range. Hits and correct rejections for each session (i.e., 34 trials) were summarised in the form of graphs using Microsoft® Excel for Mac 2016.

### **2.3 Results**

All five dogs met the termination criterion (i.e., four consecutive sessions above 80% across positive and negative samples) within 65 sessions (range = 4-62; mean = 35.4; SD = 22.51; Figures 5-9). Each dog displayed a higher overall hit rate than correct rejection rate, meaning that dogs were indicating positive on most trials and rejecting only a minority of the negative samples. The correct rejection rates of each dog generally increased over the course of sessions, with the exception of

Luna (Figure 5) whose data remained relatively variable; and Louis (Figure 9), who displayed a high level of accuracy immediately after training.

In order to facilitate data collection and maximise efficiency, no dog moved on to Experiment 2 until all five had acquired the odour discrimination. Consequently, more data is available for some dogs (i.e., Luna, Vincent, and Ruby) than others (i.e., Mica and Louis).

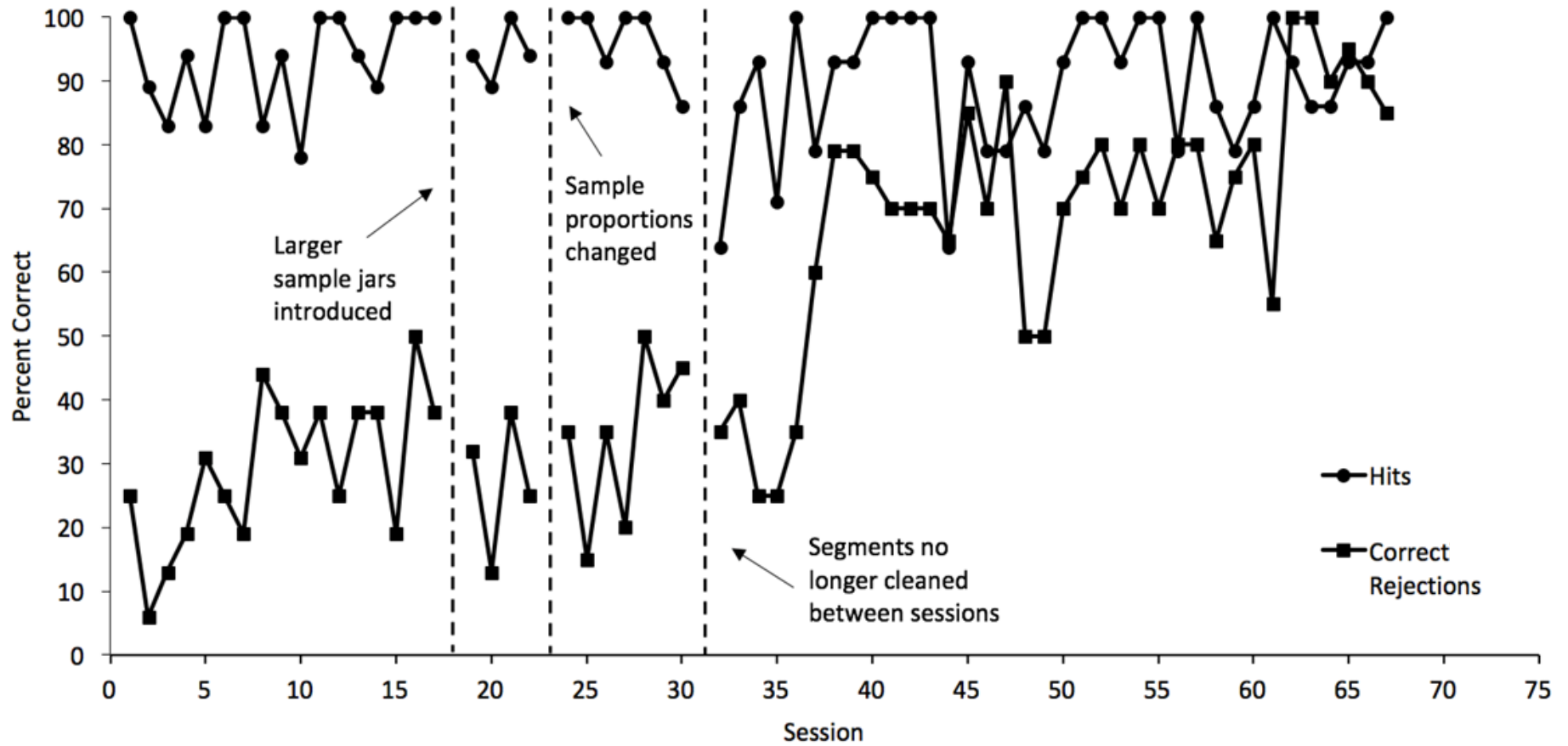


Figure 5. Luna's performance across positive (i.e., koi carp) and negative (i.e., no-fish) samples during Experiment 1.

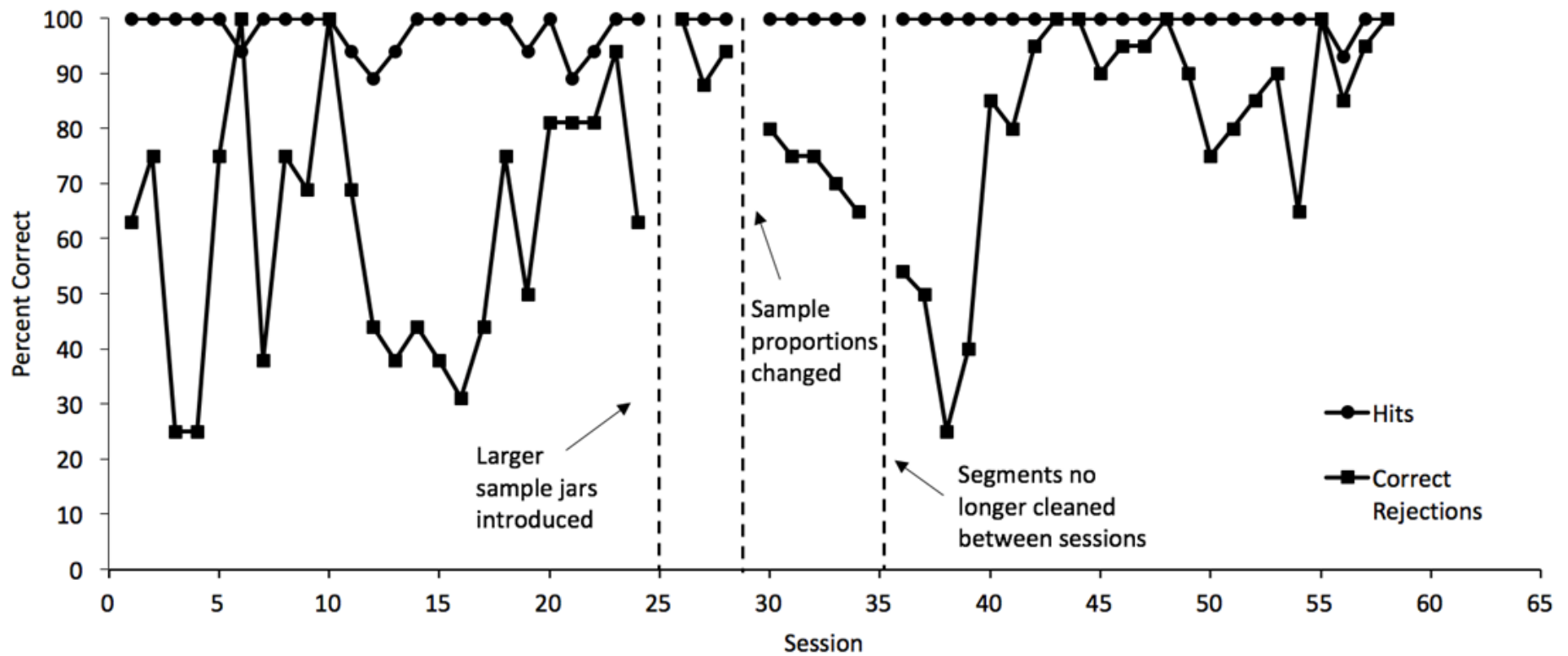


Figure 6. Vincent's performance across positive (i.e., koi carp) and negative (i.e., no-fish) samples during Experiment 1.

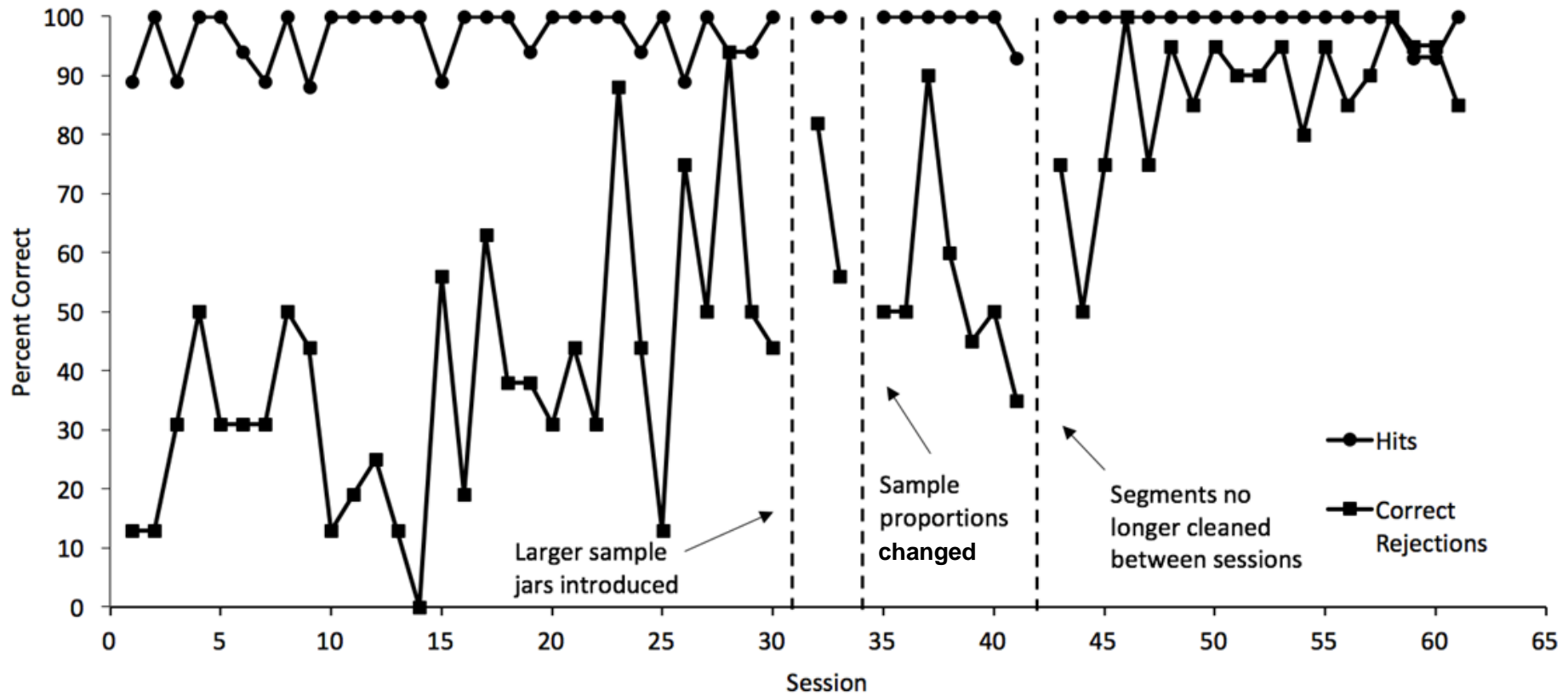


Figure 7. Ruby's performance across positive (i.e., koi carp) and negative (i.e., no-fish) samples during Experiment 1.

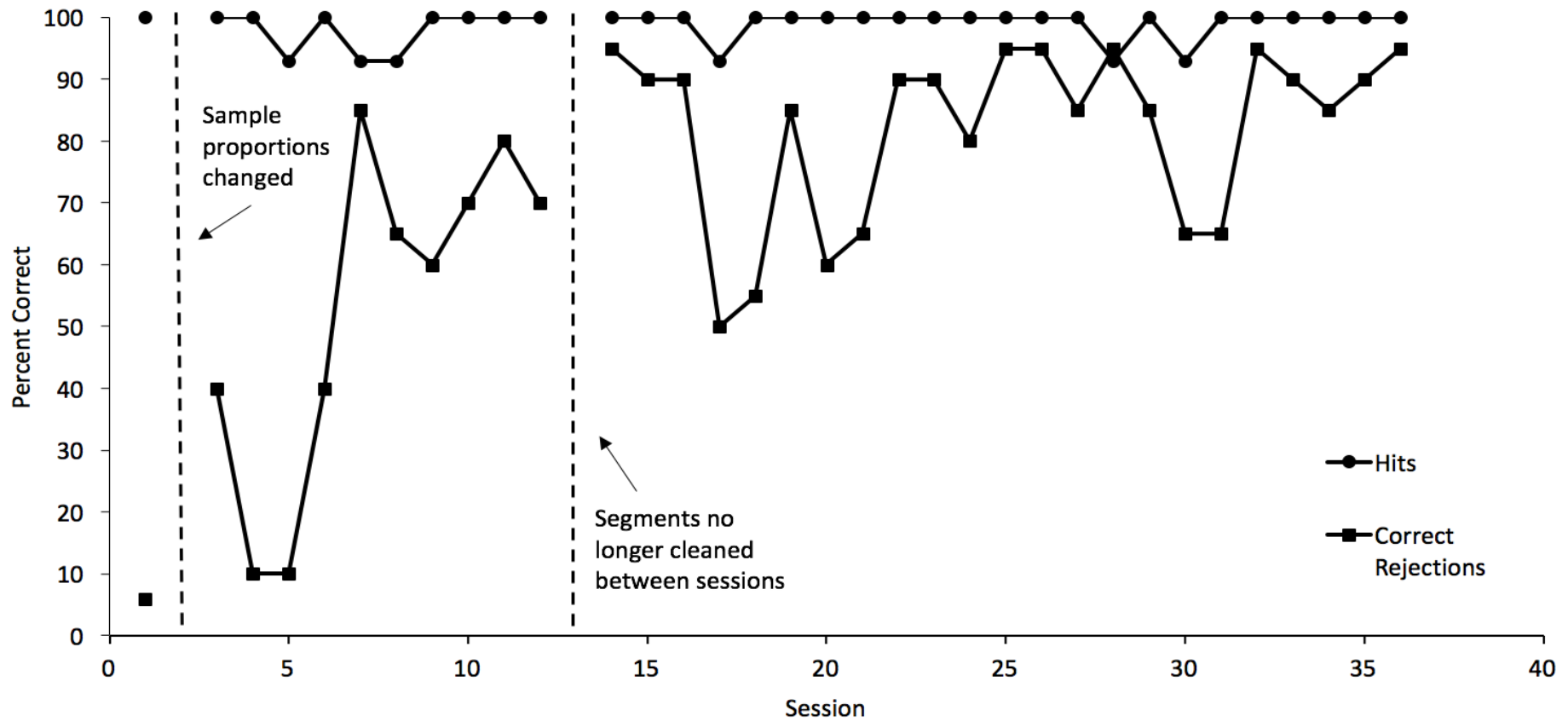


Figure 8. Mica's performance across positive (i.e., koi carp) and negative (i.e., no-fish) samples during Experiment 1.

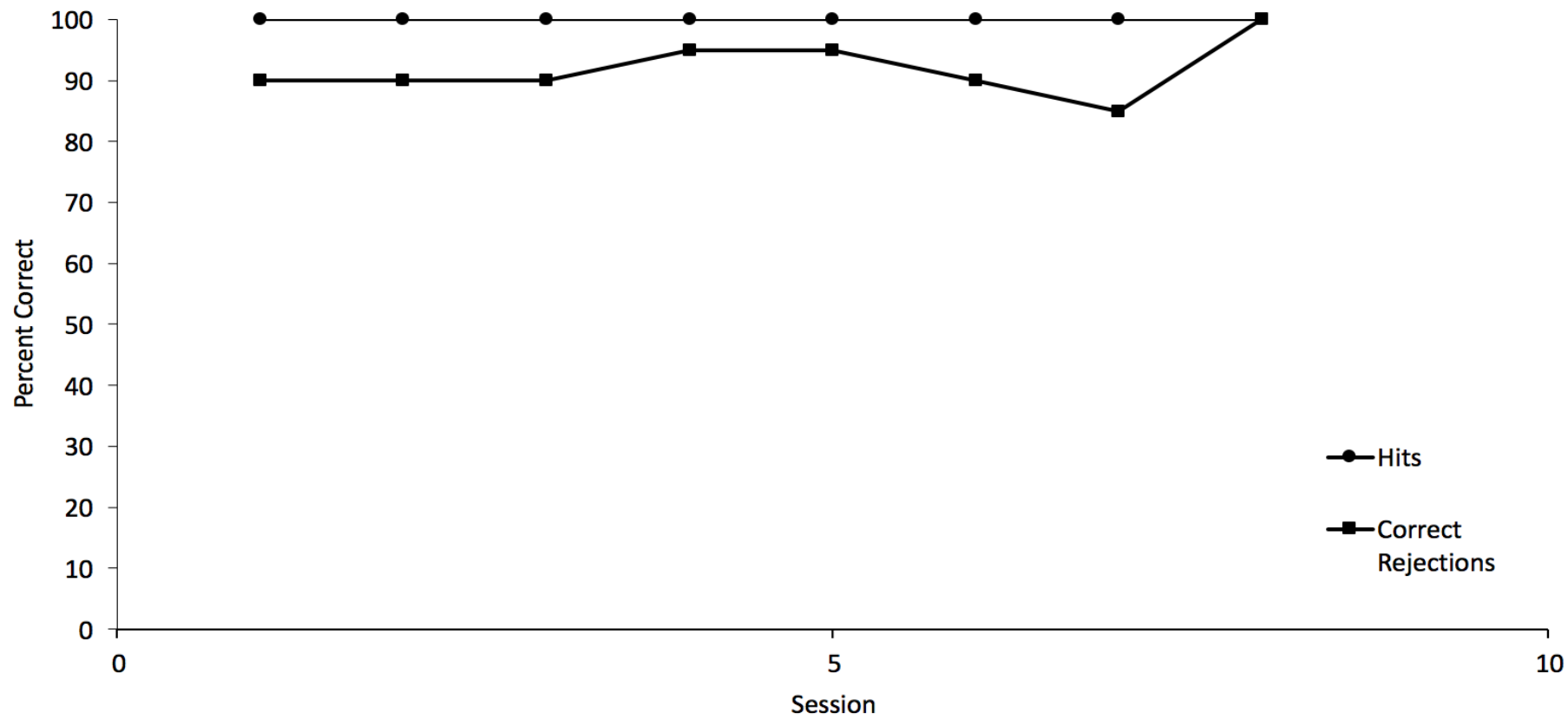


Figure 9. Louis' performance across positive (i.e., koi carp) and negative (i.e., no-fish) samples during Experiment 1.

## 2.4 Discussion

All five dogs were able to discriminate between water samples that had and had not contained koi carp with levels of accuracy well beyond what could occur by chance. This is not entirely unexpected given their success at detecting submerged human cadavers (e.g., Koenig, 2000). However, this is the first published research that has demonstrated dogs' ability to detect a freshwater fish species from water samples.

The individual data for each dog are relatively variable. The high standard deviation score (22.51) and large range (4-62) derived from the number of sessions taken to complete the experiment indicates that there was a large amount of disparity among individual dogs in terms of the number of sessions required to complete the experiment. During the first part of the experiment, the proportions of positive/negative samples were approximately 50/50, and dogs were likely indicating most samples as positive due to a 50% probability of obtaining a reinforcer. Once sample proportions were adapted so that the probability of a positive trial was approximately 40%, most dogs' accuracy rates increased. This finding could also be due to practice effects; however, most dogs had already had significant practice during training and post-training trials. In addition, data from Mica and Louis (i.e., Figures 8 and 9, respectively) suggest that practice had less of an effect on overall detection accuracy than the adjustment of sample proportions. This is evidenced by the fact that both of these dogs completed the experiment in far fewer sessions than the other three subjects.

As is the case in any study involving scent detection, it is possible that negative samples were contaminated during some of the sessions. For example, it was observed throughout the experiment that several dogs consistently indicated positive on the same negative samples. This may explain the high level of data variability during the first half of the experiment prior to the use of separate segments for each sample type. Once separate segments were allocated for each sample type and segments were no longer cleaned between sessions, most dogs' accuracy increased considerably. These data support the hypothesis that there was likely some degree of contamination of negative segments, as the primary issue was false alarms (not misses) prior to this alteration.

Figure 5 shows that Luna's accuracy during negative trials dropped shortly after the proportions were changed. It is unclear exactly why this happened, however, session notes from this time suggest that Luna was unwell for several weeks during this period. It is possible that this adversely affected her ability to discriminate between sample types.

Figures 8 and 9 show the performance of Mica and Louis, respectively. Although Mica was the first participant recruited in this study, she was absent (due to being in oestrus) for several periods throughout the year. As such, she began Experiment 1 after the larger sample jars were introduced, and completed only one session before sample proportions were adapted. Louis was the final dog recruited into the study, and therefore missed these methodological adjustments. Given that both dogs began the experiment later (i.e., after changes to the initial methodology were made to encourage discrimination), it is likely that their ability

to master the odour discrimination task was significantly increased. For example, Louis met the termination criterion after just four sessions during Experiment 1 (Figure 9). It is possible that, were the experiment to be completed again from the beginning with adapted sample proportions, large sample jars, and the use of separate segments for each sample type, the other dogs (Luna, Ruby, and Vincent) would have completed the experiment in much fewer sessions.

# Chapter Three

## Experiment 2: Dilution of positive samples

---

### 3.1 Introduction

Experiment 1 demonstrated that dogs are able to discriminate water samples that have or have not contained koi carp. However, these samples were collected from aquaria that contained fish biomass concentrations significantly higher than those normally found within the natural environment. For example, a recent study in a ‘typical’ inland lake (i.e., Lake Ohinewai in the Waikato region of New Zealand) estimated the abundance of koi carp at approximately 94 kg/ha (Tempero & Hicks, 2017), while those in the aquaria used in Experiment 1 amounted to approximately 373,737 kg/ha (see section 3.2.4). Previous research has suggested that the threshold for ecosystem degradation in shallow eutrophic lakes is in the range of 50-100 kg/ha (Badiou & Goldsborough, 2010; Bajer et al., 2009). As such, while the results of Experiment 1 lend credence to the hypothesis that dogs can detect fish through the medium of water, they reveal little about the real-world applicability of detection dogs for conservation purposes in this area. The aim of this experiment was to determine whether dogs are able to detect the presence of koi carp at dilutions similar to those found within the natural environment.

### 3.2 Methods

#### 3.2.1 Subjects

All five dogs that participated in Experiment 1 also participated in this experiment.

The same four fish used in the previous experiment were also used.

### **3.2.2 Materials and apparatus**

The materials and apparatus remained the same as Experiment 1 with the exception that 10 mL syringes were used to measure fish water for dilutions lower than 50%, and auto pipettes were used for dilutions below 6.25%.

### **3.2.3 Sample collection**

The sample collection procedure remained the same as in the previous experiment.

### **3.2.4 Sample preparation**

In order for the dogs' performance in this experiment to be relevant under real-world conditions, the lower-limit dilution of the water samples should fall somewhere below the 50 kg/ha threshold. True kg/ha calculations are not associated with a standard depth, so the depth of a 'typical' lake in the Waikato region (i.e., Lake Ohinewai with an average depth of 2.53 m) was used as a basis for comparison to convert the kg/ha into kg/L for the purposes of this study. Calculations were performed using data from a recent biomass survey conducted in Lake Ohinewai (Tempero & Hicks, 2017) and those based on the dilution of the aquaria in the present study.

First, in order to determine the fish biomass concentrations of the aquaria, the total weight of fish in the tank with the lowest weight value (1.7 kg) was divided by the total sampling volume of the tank (115 L), yielding a dilution factor of  $1.48^{-02}$  (i.e., 0.0148 kg/L). To determine the target dilution for this study (i.e., the level of fish biomass that would be ideally monitored in a real-world scenario), serial dilutions were applied (hypothetically) to the dilution factor of the tanks. For

example, a serial dilution of 10 yields a dilution factor of  $1.48^{-03}$ , a serial dilution of 100 yields a dilution factor of  $1.48^{-04}$ , and so on. These were applied in sequence until a suitable dilution was found. This was achieved using the formula:

$$(a \times b) / (c \times [\text{serial dilution}])$$

Where “a” is the carp biomass density (kg) of Lake Ohinewai at 50 kg/ha, “b” is the lake area (ha), and “c” is the total water volume ( $\text{m}^3$ ). Using a serial dilution of 1000, the equation would appear as follows:

$$(50 \times 16.8) / (424,895 \times 1000)$$

This calculation yields a dilution factor of  $1.98^{-06}$  (i.e., 0.00000198 kg/L), which is far greater than that of the undiluted aquaria water used in Experiment 1 (i.e.,  $1.48^{-02}$  or 0.0148 kg/L). In order to compare the biomass densities of koi carp in Lake Ohinewai to those of the aquaria in the present study, the following calculation was used:

$$(a / b) \times 50$$

Where “a” is the dilution factor of the tanks and “b” is the dilution factor of Lake Ohinewai at 50 kg/ha:

$$(1.48^{-02} / 1.98^{-06}) \times 50$$

This calculation yields an equivalent biomass density of approximately 373,737kg/ha in the aquaria used in Experiment 1. It was discovered that a serial dilution of 10,000 (i.e.,  $1.48^{-06}$ ) would generate a biomass density of <50 kg/ha. The same calculation, as above, was used to demonstrate this using the new value (i.e.,  $1.48^{-06}$ ):

$$(1.48^{-06} / 1.98^{-06}) \times 50$$

This calculation yields an equivalent biomass density of 37.4 kg/ha. A serial dilution of 10,000 of the tank water in the present study was therefore selected as the positive water sample dilution.

Negative samples were prepared according to the procedure described in section 2.2.6. Positive samples were initially prepared using 50% water from aquaria containing koi carp 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 until a serial dilution of 10,000 was reached (i.e., 0.098%, or 0.098 mL in approximately 99 mL of control water). It was thought that halving positive sample dilutions, rather than diluting them logarithmically, would allow the dogs to more readily adjust to the new dilution.

### **3.2.5 Cleaning and sample control**

In addition to the precautions described in section 2.2.9, several other considerations were made. For example, if multiple dogs were present at the lab and each was working with a different dilution, sessions with the dog who had reached the lowest dilution were always run first. This was to ensure that the concentrations of fish-related volatiles contained within each of the segments did not exceed the dilution at which dogs were currently working. If this was not possible to arrange, as in the case of afternoon dogs performing at lower dilutions than morning dogs, each of the 17 segments was cleaned with disposable paper towels soaked in a 70% isopropanol solution to remove residual odours and “reset” the apparatus for the next dog.

### **3.2.6 Experimental procedure**

The procedure used for this experiment was the same as that described in 2.2.9. The termination criterion for moving to subsequent sample dilutions was also the same as that used in Experiment 1 (i.e., four consecutive sessions above 80% accuracy across each sample type [positive and negative]). A criterion for withdrawal from the experiment was also set to ensure that dogs did not remain on the same dilution for too long without progressing. In this case, if dogs had not achieved the first criterion within 25 sessions, the experiment was terminated and dogs were withdrawn from participating in subsequent experiments.

### **3.2.7 Data analysis**

Data were analysed using descriptive statistics to derive the average accuracy rates for each sample type (i.e., hits and correct rejections) across individual sessions (i.e., 34 trials). These data were then summarised in the form of graphs using Microsoft® Excel for Mac 2016.

## **3.3 Results**

Three of the five dogs (Luna, Ruby, and Mica) in this experiment were able to detect the presence of koi carp at dilutions of 0.098% with levels of accuracy well beyond what could occur by chance (Figures 10, 12, and 13). Two dogs (Vincent and Louis) were unable to progress past the 12.5% dilution and were withdrawn from the study (Figures 11 and 14).

The dogs typically displayed higher overall hit rates (i.e., Luna, 98%; Vincent, 87%; Ruby, 94%; Mica, 96%; and Louis, 96%) than correct rejection rates

(i.e., Luna, 84%; Vincent, 74%; Ruby, 86%; Mica, 84%; and Louis, 68%). This suggests that dogs were biased toward positive responding.

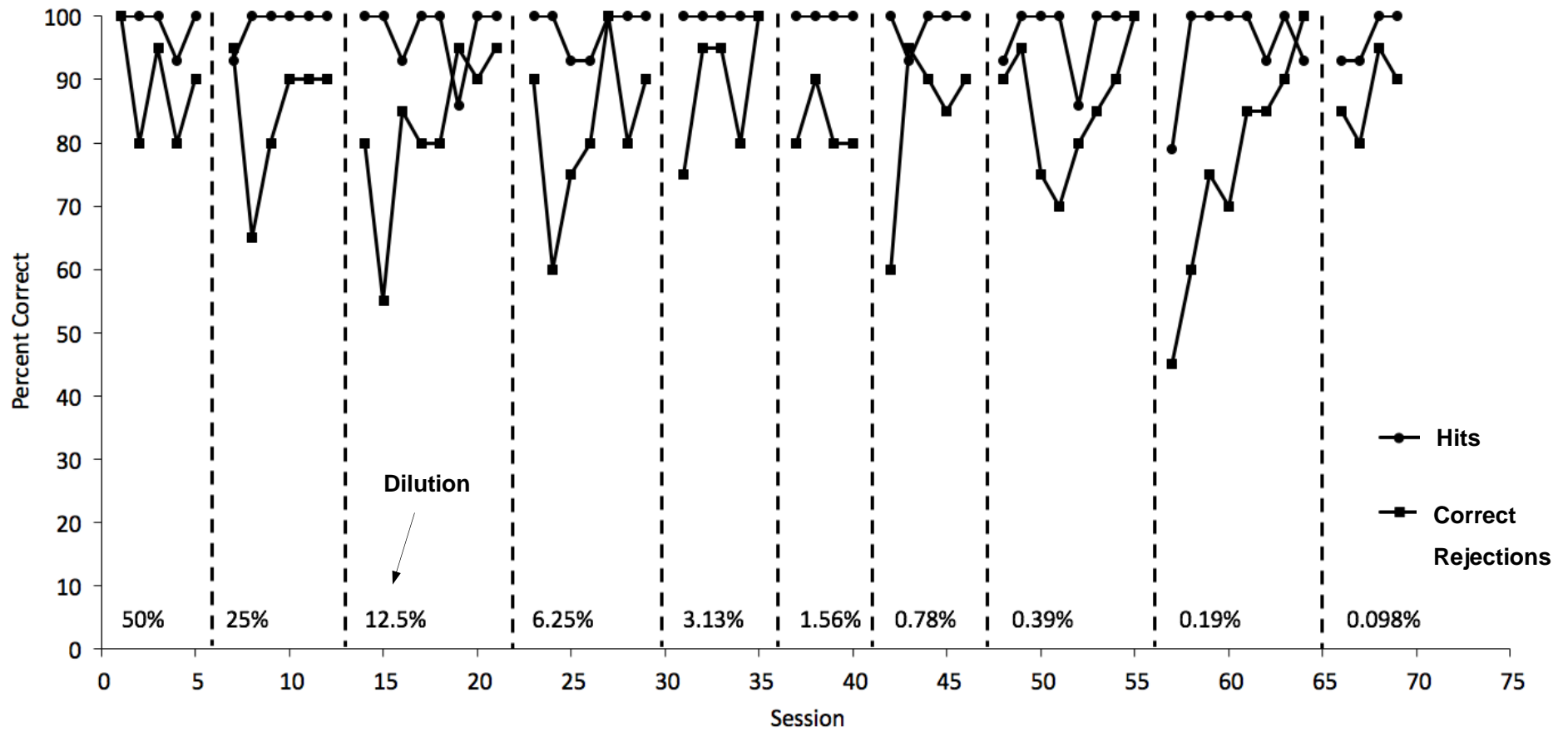


Figure 10. Luna's performance across positive (i.e., koi carp) and negative (i.e., no-fish) sample dilutions during Experiment 2.

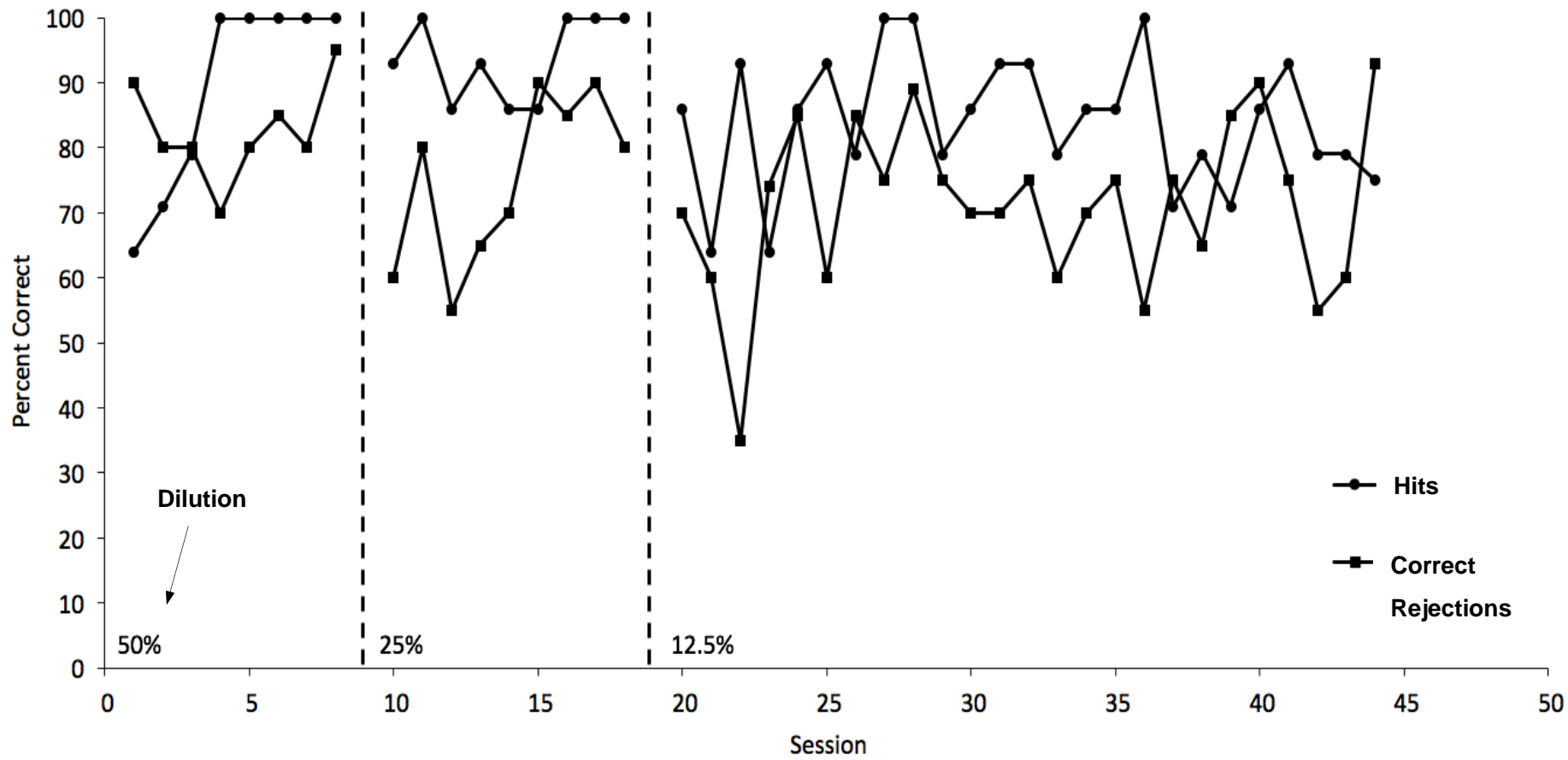


Figure 11. Vincent's performance across positive (i.e., koi carp) and negative (i.e., no-fish) sample dilutions during Experiment 2.

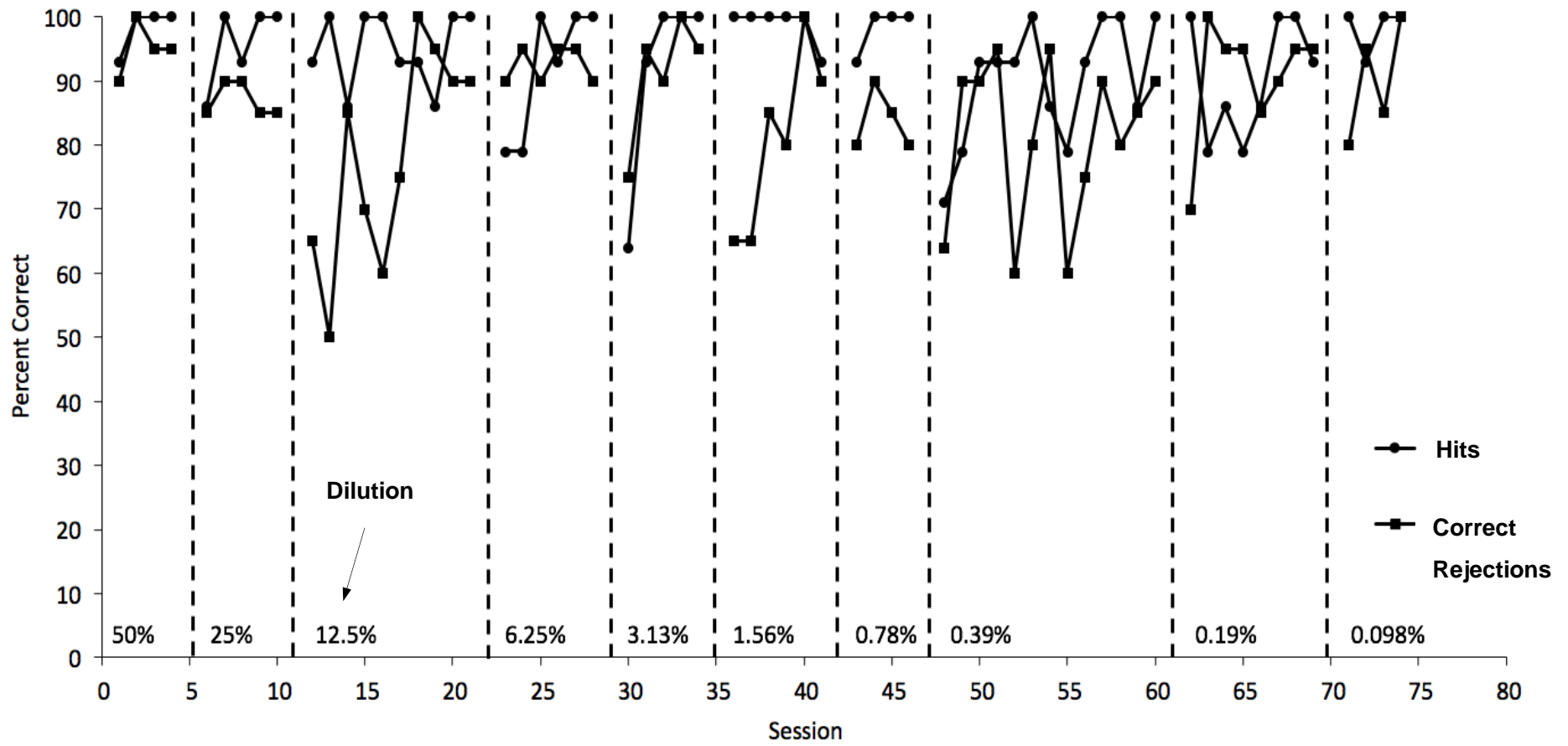


Figure 12. Ruby's performance across positive (i.e., koi carp) and negative (i.e., no-fish) sample dilutions during Experiment 2.

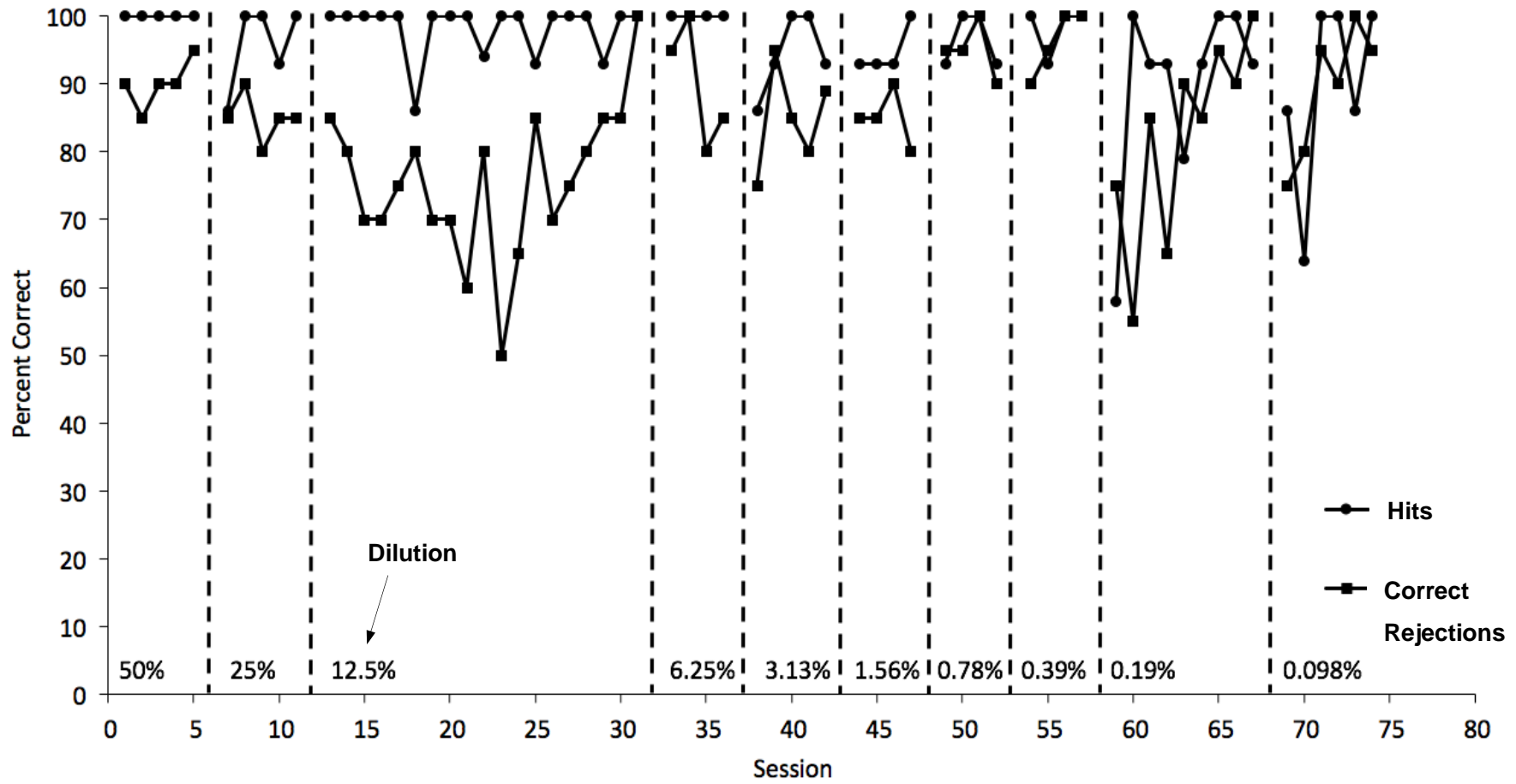


Figure 13. Mica's performance across positive (i.e., koi carp) and negative (i.e., no-fish) sample dilutions during Experiment 2.

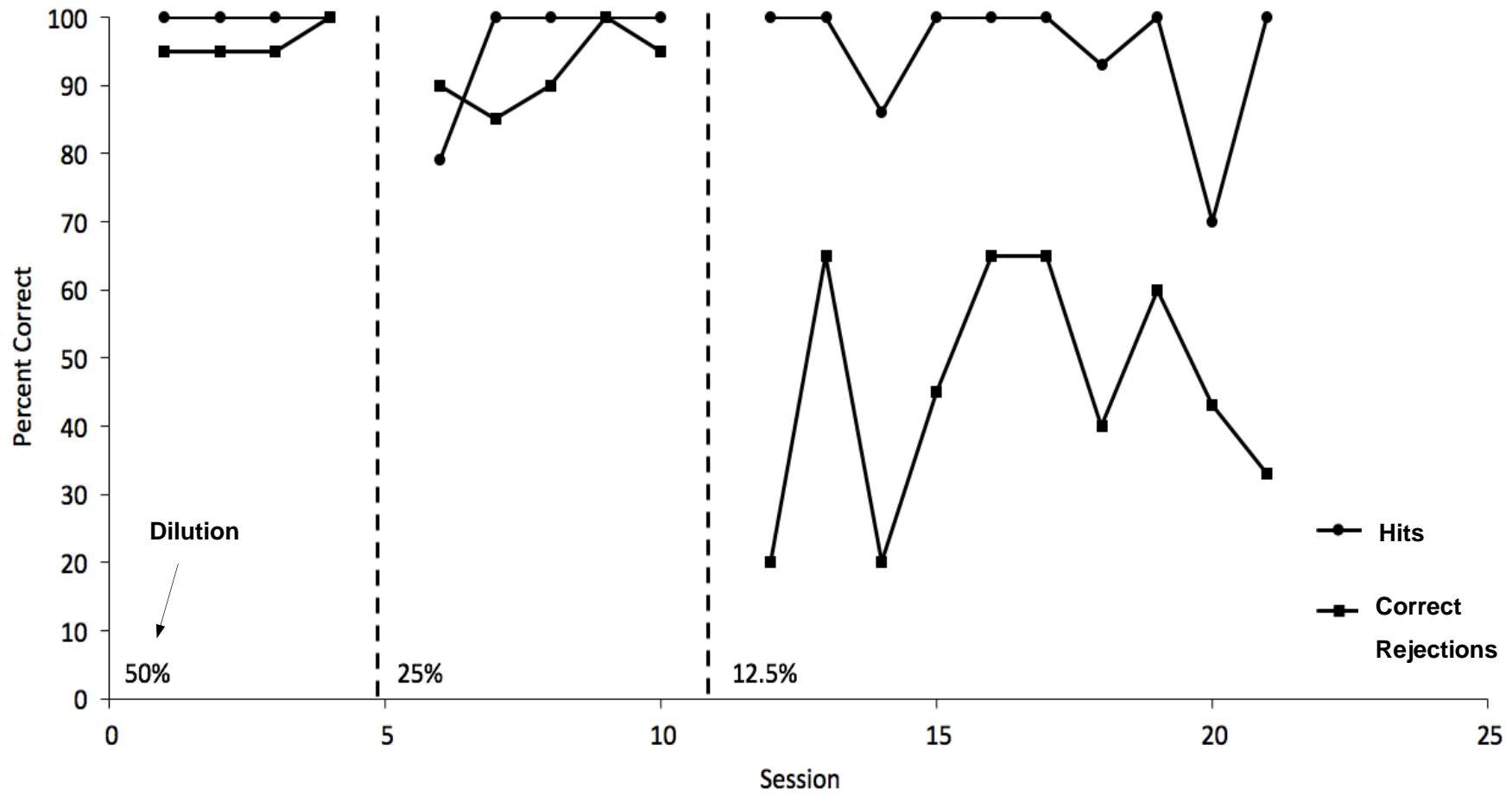


Figure 14. Louis' performance across positive (i.e., koi carp) and negative (i.e., no-fish) sample dilutions during Experiment 2.

### **3.4 Discussion**

The results of this experiment demonstrate that dogs are able to detect the presence of koi carp from water samples at dilutions that are biologically significant (i.e., similar to those found within the natural environment). Three of the five dogs (i.e., Luna, Ruby, and Mica) in this experiment were able to detect koi carp from positive water samples diluted to 0.098% of their original state. While previous research has suggested that dogs are capable of detecting lower limit concentrations of n-amyl acetate of approximately one part per trillion (e.g., Walker et al., 2006), the present study did not use a chemical of similar aromaticity, which precludes any direct comparisons. Conventional detection methods (e.g., electrofishing, passive netting, visual observation) are often unable to detect fish in water that is deep (Banks & Hogg, 2015; Magnuson et al., 1994) or sparsely populated by the target species, as fish must first be observed or captured. Dogs may prove to be a useful tool in this area given their success at detecting koi carp in this experiment.

The performance of each dog typically dropped during the first session in which they were exposed to a reduced sample dilution, following mastery of the previous dilution. Interestingly, four out of five dogs demonstrated a large drop in accuracy 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 (i.e., 25% and 12.5%).

It is possible that, had further dilutions been prepared, all three dogs that successfully completed this experiment would have been able to detect koi carp at much lower concentrations. However, the utility of this is questionable. Koi carp, like many other invasive species, are extremely difficult to eradicate completely and do not represent a significant threat when present in low numbers (Collier & Grainger, 2015). For this reason, working to detection limits of 0.098% (i.e., an equivalent biomass concentration of 37.4 kg/ha) was thought to be sufficient in that it would allow for the detection of koi carp before they are able to reach the threshold for environmental degradation (i.e., 50-100 kg/ha; Badiou & Goldsborough, 2010; Bajer et al., 2009).

Two dogs were withdrawn from this study during Experiment 2. Vincent's performance (Figure 11) dropped such that he was not able to advance to subsequent dilutions after meeting the withdrawal criterion (i.e., 25 sessions at the same dilution). Louis (Figure 14) began to complete fewer and fewer sessions each day over the course of the experiment and could not be motivated to enter the experiment room or work; he was subsequently withdrawn from the experiment. It was initially hypothesised that the dry dog kibble no longer functioned as a suitable source of reinforcement for Louis given the increasing difficulty of the task. However, it was later discovered that Louis had developed allergies and was required to consume a special diet to control his gastric problems. This illness may have accounted for the poor performance during his last few sessions, and it is possible that, had he not become ill, the high accuracy that he had displayed up until this point would have continued across subsequent dilutions.

## Chapter Four

### Experiment 3: Discrimination among distantly-related fish species

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#### 4.1 Introduction

Experiment 2 demonstrated that dogs are able to detect koi carp at concentrations similar to those found within the natural environment. While this represents a useful addition to the current literature, it begs the question: Are dogs simply detecting a general “fish” odour (as compared to a neutral odour), or do different species of fish produce their own unique VOC signatures? It is known that multiple species of freshwater fish often co-occur in the same habitat (Collier & Grainger, 2015). Koi carp, for example, live alongside many other species of fish, both distantly- (i.e., catfish, *Ameiurus nebulosus*) and closely- (i.e., goldfish, *Carassius auratus*) related. Consequently, if this procedure is to be considered for applied conservation work, dogs must be able to discriminate between several species of fish or risk generating unreliable data. Similar species are taxonomically alike and share a larger proportion of DNA, meaning that they are often difficult distinguish from one-another; while distantly-related species tend to be more easily discriminated (Smart et al., 2016). As such, it was hypothesised that a distantly-related fish species (i.e., catfish) would be more readily detected by the dogs’ olfactory receptors. Bearing this hypothesis in mind, the present experiment aimed to determine whether dogs could discriminate koi carp from one of its distant relatives (i.e., catfish).

## **4.2 Method**

### **4.2.1 Subjects**

#### **4.2.1.1 Dogs**

Three of the five dogs (Luna, Ruby, and Mica) from the previous experiments also participated in Experiment 3.

#### **4.2.1.2 Fish**

Two wild koi carp present during Experiments 1 and 2, and eight wild brown bullhead catfish, participated in this experiment.

### **4.2.2 Materials and apparatus**

The materials and apparatus remained the same as in the previous experiments with the addition of a glass beaker, collection bottle, and auto-pipette attachment for the catfish water samples.

### **4.2.3 Sample collection**

Prior to beginning this experiment, the koi carp from Tank 3 were removed. The fish in this tank were selected for removal as they weighed slightly more than those in Tank 2 (i.e., 2 kg vs. 1.7 kg), and it was agreed that the lower dilution of fish would produce more conservative, and therefore more informative, data. The same koi carp were used throughout the entire study, and they appeared to have habituated to the presence of humans during cleaning and sample collection (i.e., they were observed to startle less frequently). Because of this, it was decided that one tank would provide sufficient water samples for the remainder of the study. Both Tanks 3 and 4 (which had previously been empty), were scrubbed with a

coarse Scotch-Brite™ cleaning pad and soapy water, sprayed with a 5% hydrogen peroxide (H<sub>2</sub>O<sub>2</sub>) solution, rinsed, and left with the water running for three days to flush out any remaining odours.

Tanks 3 and 4 were each populated with approximately four brown bullhead catfish (1.8 kg per tank). Catfish have sharp spines that protrude from their dorsal and pectoral fins (Collier & Grainger, 2015), and have been known to injure nearby fish when agitated (G. Tempero, personal communication, 2017). In order to ensure the welfare of the catfish, two tanks were used so that each tank would be cleaned and sampled from only once per week, which would minimise fish disturbance and, therefore, the potential injury of conspecifics.

Samples were collected according to a similar method to that described in section 2.2.5, with the exception that catfish samples were collected after the no-fish samples and before the koi carp samples with disposable gloves changed between the handling of each sample type. Catfish water was transported in a glass bottle in the same backpack as the koi carp water, however, each of these bottles were sealed, dried on the exterior, and enclosed within separate plastic bags to avoid cross-contamination.

#### **4.2.4 Sample preparation**

Samples were prepared according to the method described in section 2.2.6 using the lowest dilution from Experiment 2 (i.e., 0.098%) for koi carp and catfish samples. The sample proportions that were presented to dogs in this experiment were seven koi carp (positive) water samples, five catfish (control) water samples, and five no-fish (control) water samples. These proportions were used to ensure

the same rate of reinforcement per session as previous experiments, which, if adjusted, could impact the dogs' accuracy rates. Catfish samples were marked with a single rectangular adhesive label placed on the base of the sample jar, and catfish segments were marked with an adhesive label that ran horizontally across the top of the segment, to differentiate them from the other sample types.

#### **4.2.5 Experimental procedure**

The experiment was run according to the method described in section 2.2.9, with one important exception. Samples containing koi carp were still treated as positive samples, however, there were now two negative sample types: catfish and no-fish. Dogs received no food regardless of their behaviour during trials with either of these negative samples, and were required to activate the limit switch on the side of the apparatus during catfish trials in addition to the no-fish trials.

A termination criterion of four consecutive sessions above 80% accuracy across both hits and correct rejections for all three sample types was initially set. However, each dog met this criterion twice. The reasons for this are described in section 4.4, below.

#### **4.2.6 Data analysis**

The data for this experiment were analysed in a similar manner to that described in section 2.2.10, with the exception that an additional graph was generated to display the combined correct rejection rates, in addition to those of each sample type.

### **4.3 Results**

All three dogs were able to successfully discriminate between the three sample types (i.e., koi carp, catfish, and no-fish), demonstrating accuracy rates higher than what would be observed given random (chance) responding. Each dog achieved the termination criterion within 21 sessions (mean = 14.3; range = 11-21; SD = 5.8; Figures 15, 17, and 19). Dogs displayed higher overall hit rates (i.e., Luna, 95%; Ruby, 93%; and Mica, 96%) than combined correct rejection rates (i.e., Luna, 79%; Ruby, 84%; and Mica, 75%), which suggests that they were biased toward positive responding.

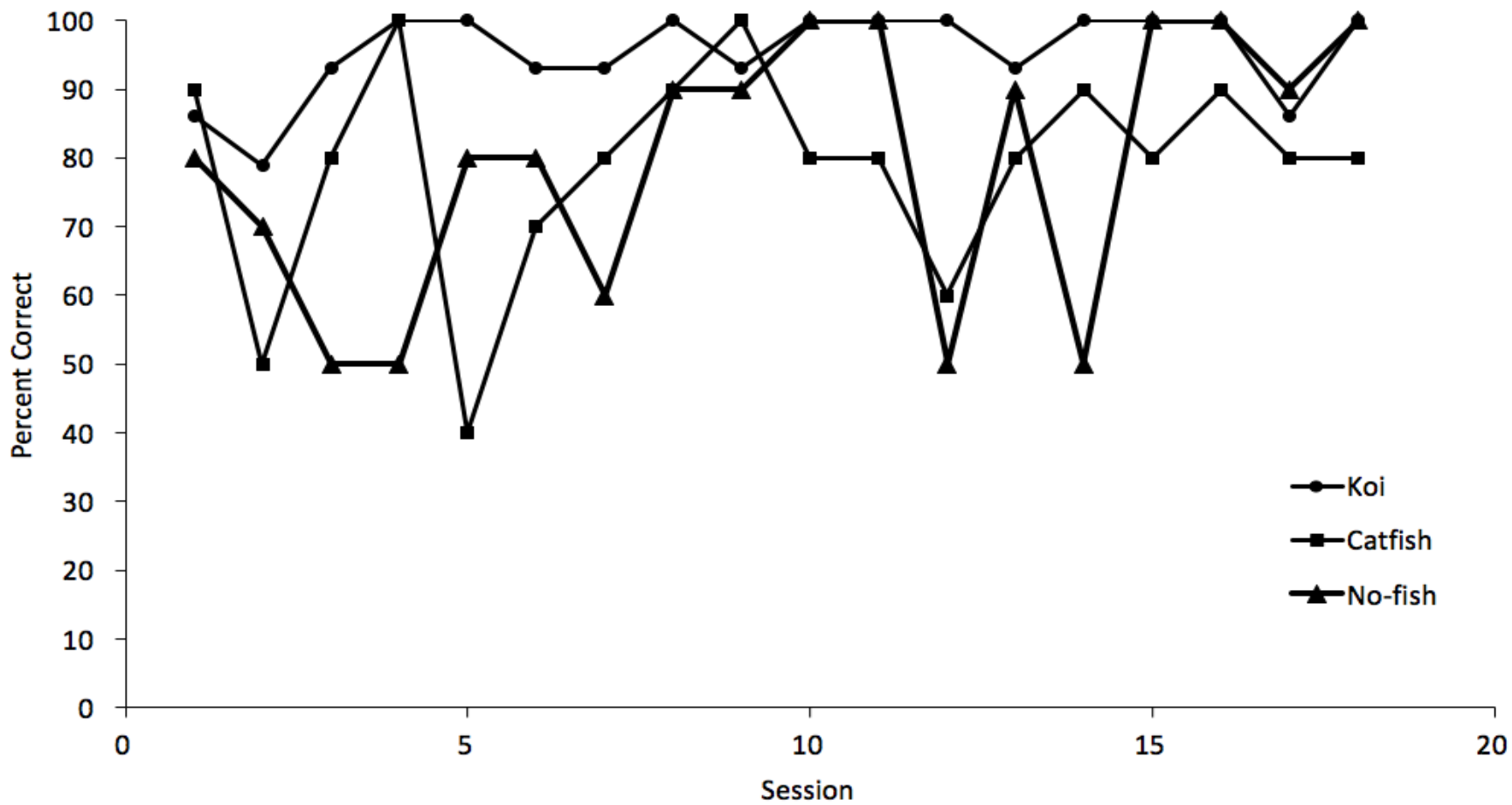


Figure 15. Luna's performance across three sample types (i.e., koi carp, catfish, no-fish).

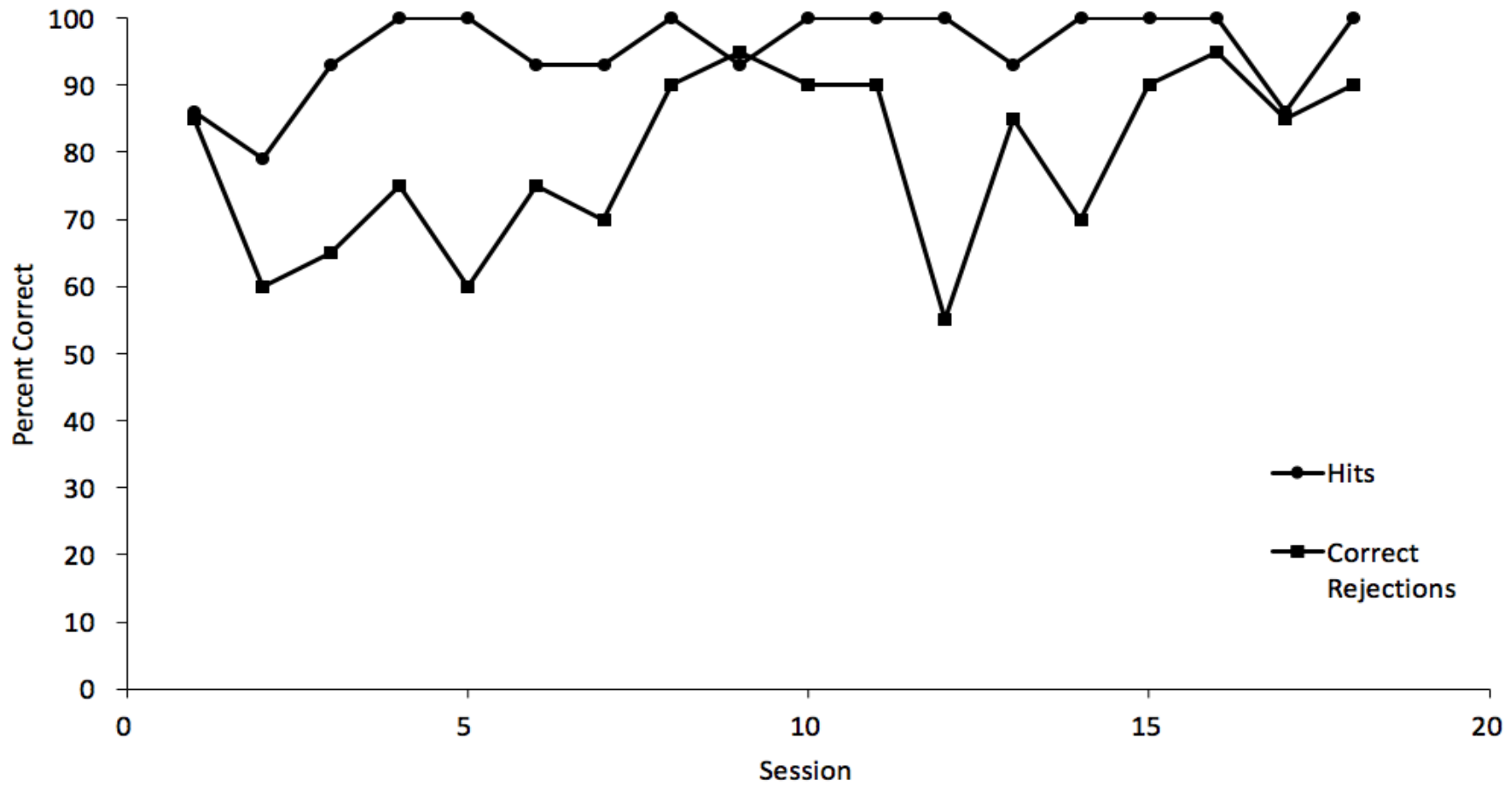


Figure 16. Hit rate plotted against the combined correct rejection accuracy (Luna).

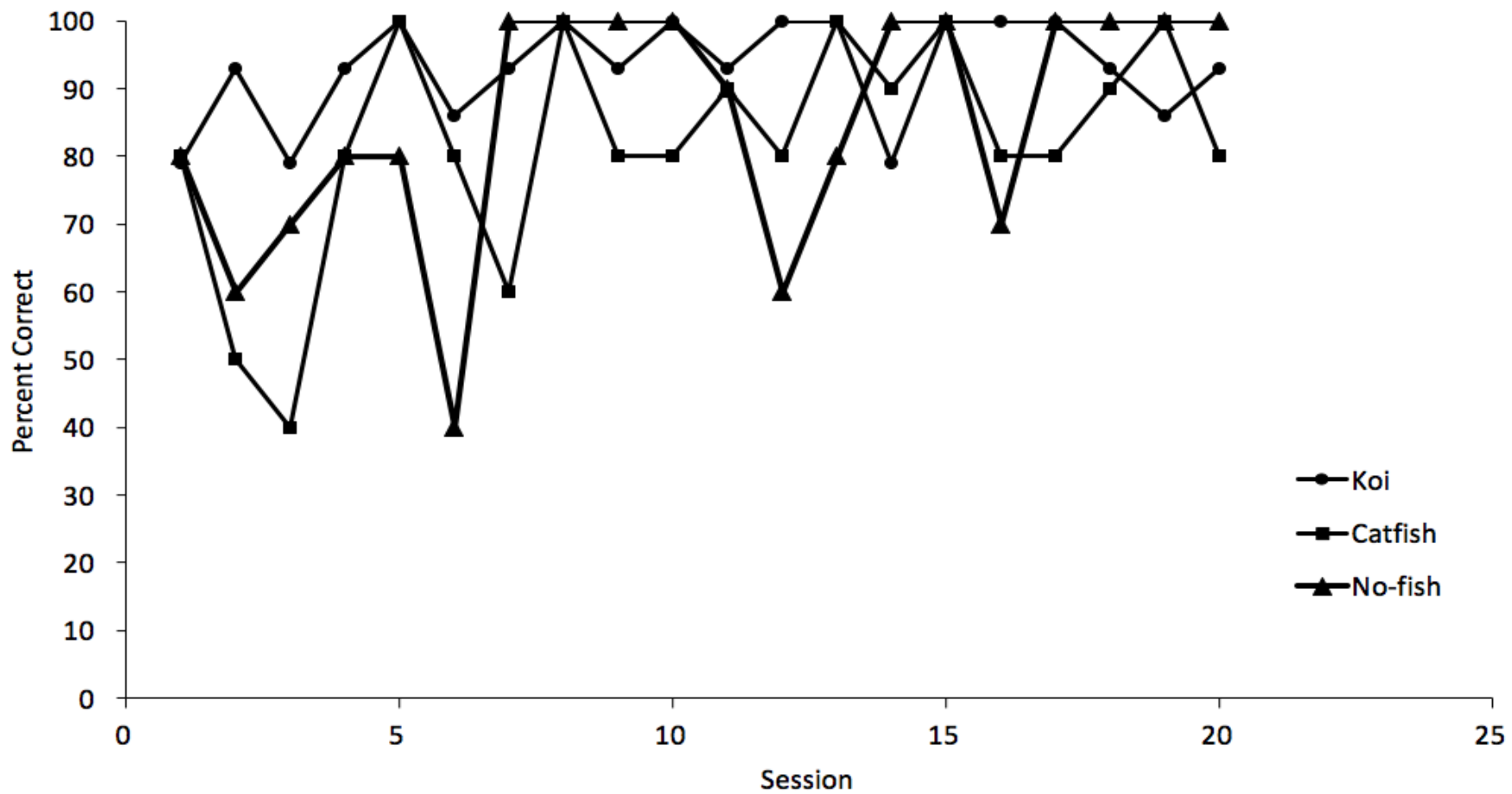


Figure 17. Ruby's performance across three sample types (i.e., koi carp, catfish, no-fish).

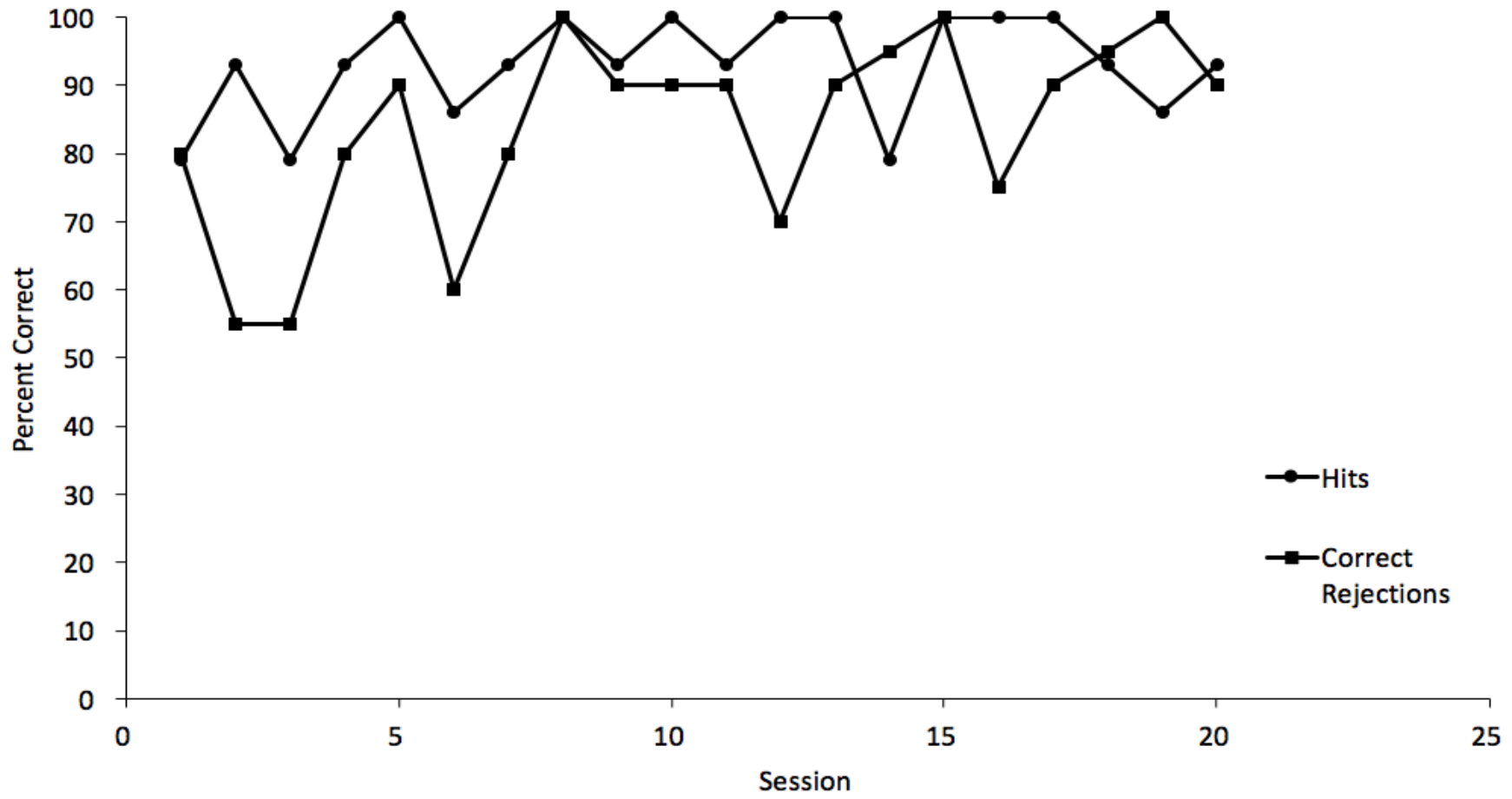


Figure 18. Hit rate plotted against the combined correct rejection accuracy (Ruby).

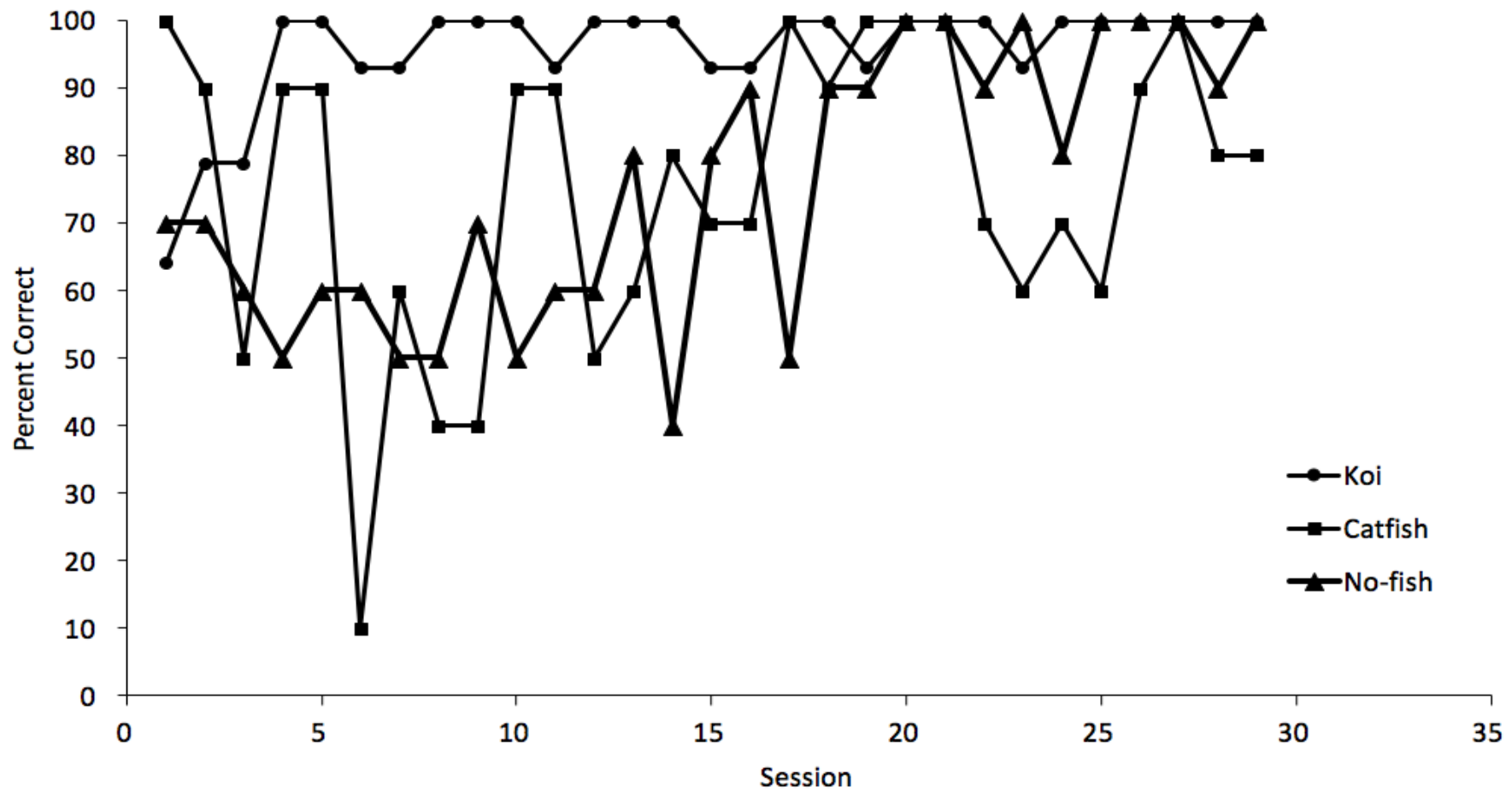


Figure 19. Mica's performance across three sample types (i.e., koi carp, catfish, no-fish).

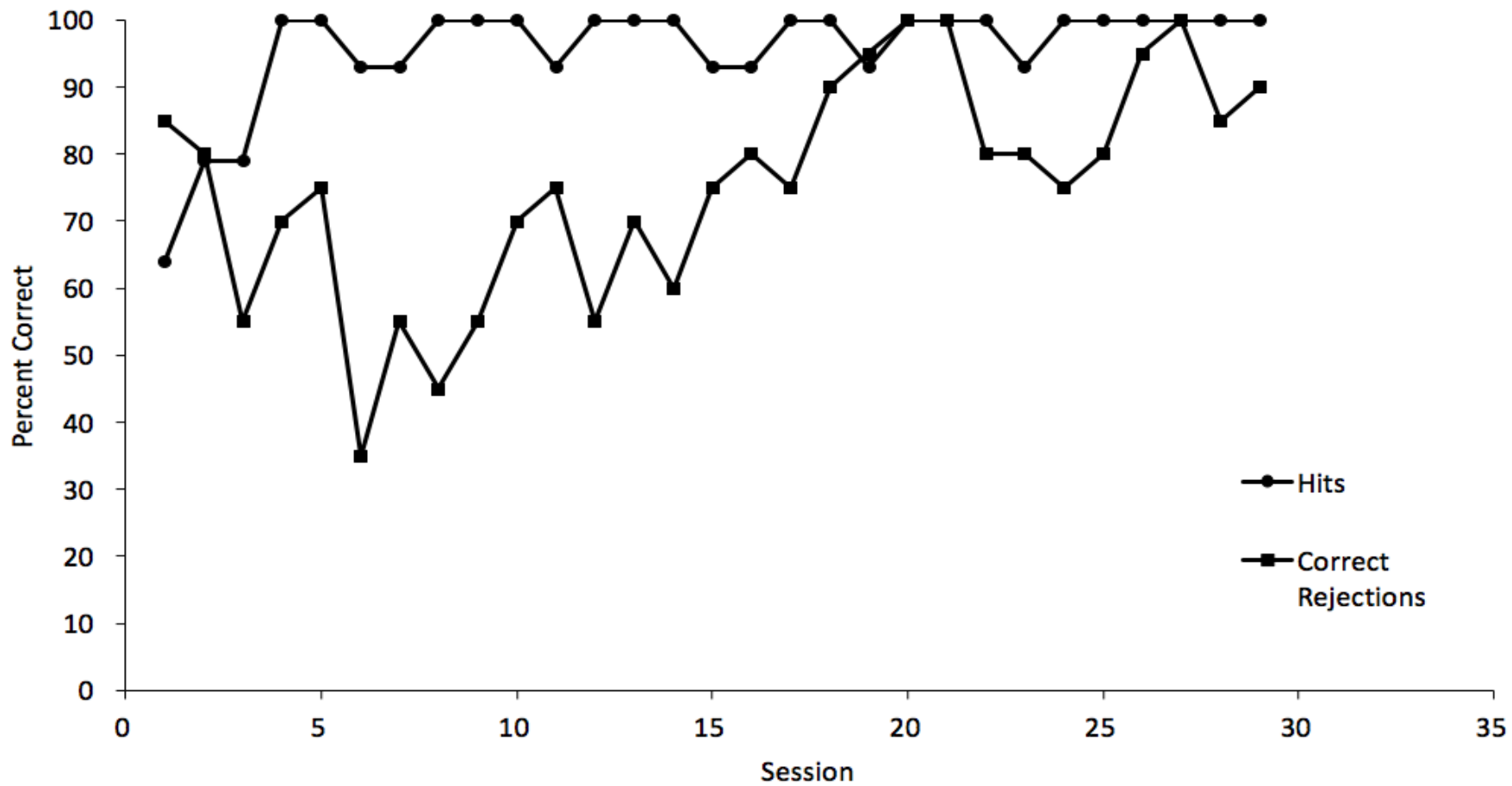


Figure 20. Hit rate plotted against the combined correct rejection accuracy (Mica).

#### 4.4 Discussion

All three dogs in this experiment successfully discriminated among koi carp, catfish, and no-fish samples. These are among the first published results to demonstrate dogs' ability to distinguish between different submerged aquatic targets.

The dogs performed slightly less accurately during negative trials in this experiment compared to Experiment 2 (i.e., Luna, 84% vs. 79%; Ruby, 86% vs. 84%; and Mica, 84% vs. 75%). This may have been due to the presence of the additional negative sample type (i.e., catfish), which dogs were required to ignore. Interestingly, higher correct rejection rates were generally observed across the catfish trials than the no-fish trials, despite the dogs having had several months of practice rejecting these no-fish samples in the previous experiments. The data appear to be more variable in this experiment than in previous experiments, although this may simply be an artefact of the sample proportions used. For example, each data point representing the correct rejection rate during previous experiments contained the average of 20 trials, while for this experiment the correct rejection rate was split into two sample types (catfish and no-fish), and these were averaged over just 10 trials during each session. However, after calculating the combined correct rejection accuracy and plotting this against the hit rate (i.e., Figures 16, 18, and 20), the data are only slightly less varied.

Both Luna and Ruby met the initial termination criterion before Mica. However, it was not possible for Luna and Ruby to begin the next experiment with a different species of fish while Mica was working towards completion of the

current experiment, so it was decided that additional data should be gathered for both dogs while catfish samples were still available. During this time, it was observed that both Luna and Ruby displayed a large drop in accuracy after less than a week of having met the termination criterion, which is concerning given its implications for an applied procedure (i.e., dogs may require significant maintenance training to achieve sufficient stimulus control over non-target odours). To better understand this drop in performance, it was agreed that each dog should complete the requirements for termination twice. All three dogs reached this criterion a second time within a fewer number of sessions (i.e., Luna, 7 vs. 11 sessions; Ruby, 9 vs. 11; and Mica, 7 vs. 21), indicating that if performance declines, it should recover relatively quickly. However, the reason for this drop in performance is unclear given that nothing had changed since the initial training.

The results of this experiment are especially useful in real-world detection scenarios in which multiple species are present, enabling dogs to accurately convey the presence of the target species. Dogs could potentially be trained to detect the presence of a number of invasive or endangered species, and to largely ignore that of extraneous or irrelevant biota, relative to the specific goals of the detection project. For example, catfish are similar to koi carp in that they are fairly resilient to adverse conditions, meaning that they easily spread and are difficult to eradicate (Collier & Grainger, 2015). Although they currently have no legal status in New Zealand, it is illegal to sell them alive (New Zealand Government, 2001) or release them once captured (New Zealand Government, 2013). Dogs could be used to detect and monitor these fish before they become invasive.

While this experiment has demonstrated dogs' ability to distinguish between koi carp and catfish (i.e., a distantly-related fish that often shares the same habitat), there is still the issue of the erroneous detection of closely-related species, which persists as a limitation of conventional species detection procedures (i.e., visual observation, netting, tagging, electrofishing, and, in the case of insufficiently specific primers, eDNA). With the ultimate purpose of conservation in mind, dogs must be able to discriminate not only among species that are distantly-related to koi carp, but also those of the same taxonomic family (i.e., Cyprinidae).

## Chapter Five

### Experiment 4: Discrimination among closely-related fish species

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#### 5.1 Introduction

Experiment 3 demonstrated that dogs are able to discriminate between distantly-related fish species that often co-occur in similar habitats. This is to say that koi carp and catfish appear to have unique VOC signatures that dogs are able to discriminate. However, in order for the procedures described in this thesis to be beneficial under real-world detection conditions, dogs must be able to detect not only distant relatives of koi carp (i.e., catfish), but also those species that are taxonomically similar (i.e., goldfish). Several studies have examined this phenomena in animals. Lin et al. (2011) found that dogs were able to detect the presence of red imported fire ants and largely ignore the presence of four other closely-related ant species (three of which were part of the same subfamily) at average hit rates of 93%. Browne et al. (2015) reported that dogs were able to distinguish among the scats of two closely-related gecko species (i.e., Marlborough green gecko and forest gecko). Well-trained detection dogs have even been reported to discriminate the scents of human monozygotic twins (i.e., twins whose DNA originates from a single zygote and are therefore biologically similar; Pinc, Bartos, Reslova, & Kotrba, 2011). Given these findings, it is not entirely unreasonable to hypothesise that dogs may also be able to discriminate the scents of biologically similar fish species. The aim of this experiment was to

determine whether dogs can distinguish the scents of koi carp from those of a taxonomically similar fish species, goldfish.

## **5.2 Methods**

### **5.2.1 Subjects**

#### **5.2.1.1 Dogs**

All three dogs (i.e., Luna, Ruby, Mica) from the previous experiment also participated in this experiment.

#### **5.2.1.2 Fish**

Two wild koi carp used during the previous experiments, and three wild goldfish, participated in this experiment.

### **5.2.2 Materials and apparatus**

The materials and apparatus remained the same as the previous experiment with the addition of a glass beaker, collection bottle, and auto-pipette attachment for the goldfish water samples.

### **5.2.3 Sample collection**

All catfish from the previous experiment were removed from Tanks 3 and 4, and Tank 3 was then cleaned and prepared according to the method described in section 4.2.3. Prior to beginning this experiment, it was discovered that several of the “goldfish” that were intended to be used were in fact koi carp-goldfish hybrids. Three purebred goldfish were identified among the remaining fish stock, weighing approximately 0.75 kg combined (i.e., less than half of that of the koi carp). It was not possible to obtain more goldfish during this time as the weather became

exceptionally poor, which restricted use of the electrofishing boat that had been assigned to capture fish for the study. To prevent further delays to the experiment, the three goldfish that were available were placed in Tank 3 and calculations were made to adjust the water volume to match the dilution of koi carp in Tank 2. The concentration of koi carp (kg/L) was calculated by dividing the weight of the fish (1.7 kg) by the sampling volume (115 L), yielding a result of 0.0148 kg of fish per litre of water. To calculate the required water volume for the goldfish in Tank 3, the weight of the fish (0.745 kg) was divided by the fish concentration in Tank 2 (0.0148 kg/L), which yielded a water volume of 50 L for Tank 3. This was used as the sampling volume (i.e., the volume of water from which samples were taken) for the goldfish. Samples were collected according to the method described in section 4.2.3

#### **5.2.4 Sample preparation**

The procedure for sample preparation during this experiment was the same as that described in section 4.2.4, except that goldfish were used in place of catfish.

#### **5.2.5 Experimental procedure**

The experiment was run according to the method explained in 4.2.5. Goldfish trials were considered negative, and, as with catfish trials, dogs received no reinforcement for correct responses. The same termination criterion as the previous experiment was set (i.e., four consecutive sessions above 80% accuracy across both hits and correct rejections for all three sample types).

#### **5.2.6 Data analysis**

Data were analysed in the same manner as described in section 4.2.6.

### 5.3 Results

All three dogs were able to successfully discriminate between the three sample types (i.e., koi carp, goldfish, and no-fish), with levels of accuracy well beyond what could occur by chance. All dogs achieved the termination criterion within 14 sessions (mean = 10; range = 5-14; SD = 4.6; Figures 21, 23, and 25).

Similar to Experiment 3, dogs displayed higher overall hit rates (i.e., Luna, 97%; Ruby, 100%; and Mica, 93%) than combined correct rejection rates (i.e., Luna, 79%; Ruby, 89%; and Mica, 79%), which suggests that they were biased toward positive responding.

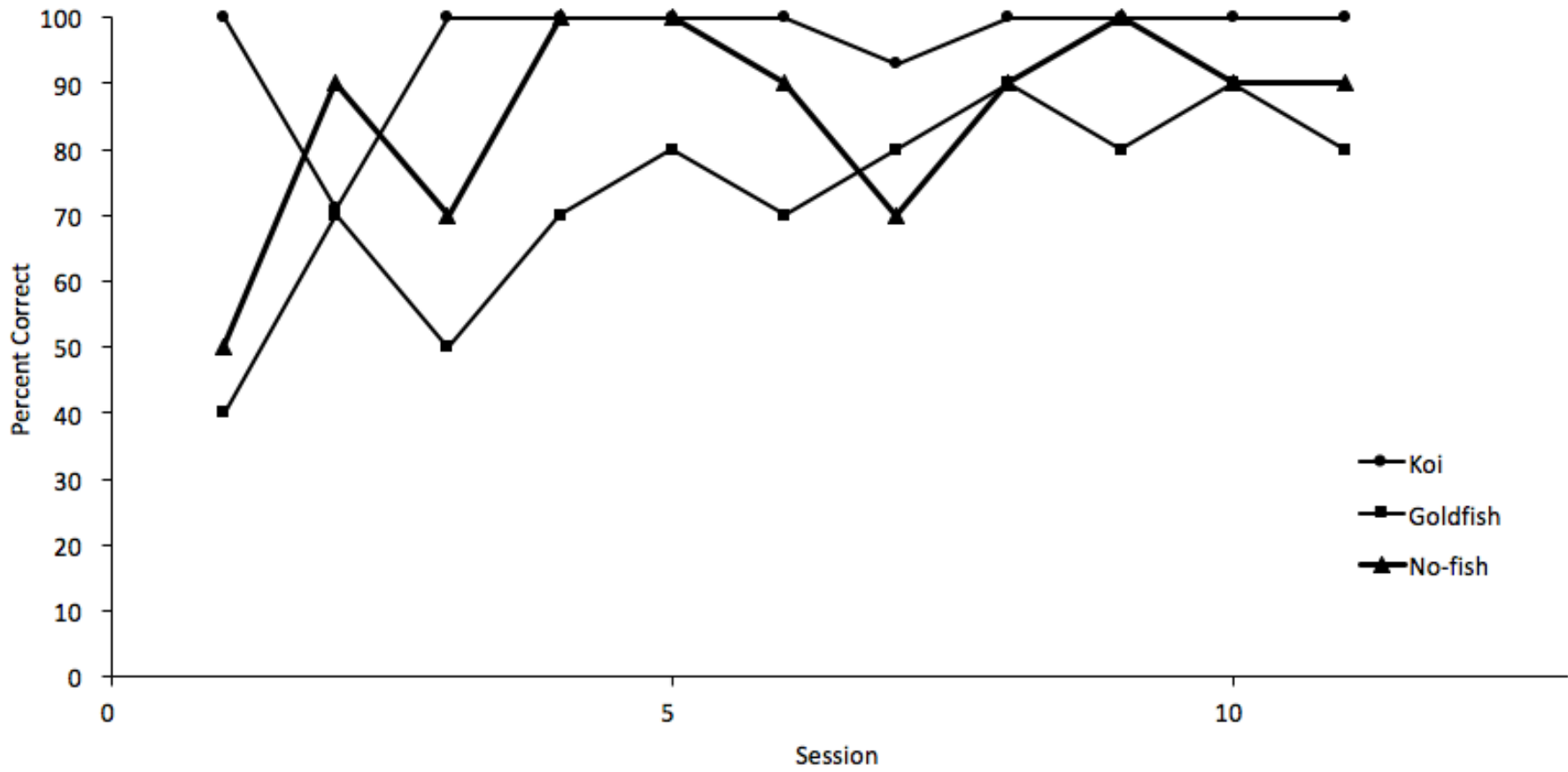


Figure 21. Luna's performance across three sample types (i.e., koi carp, goldfish, no-fish).

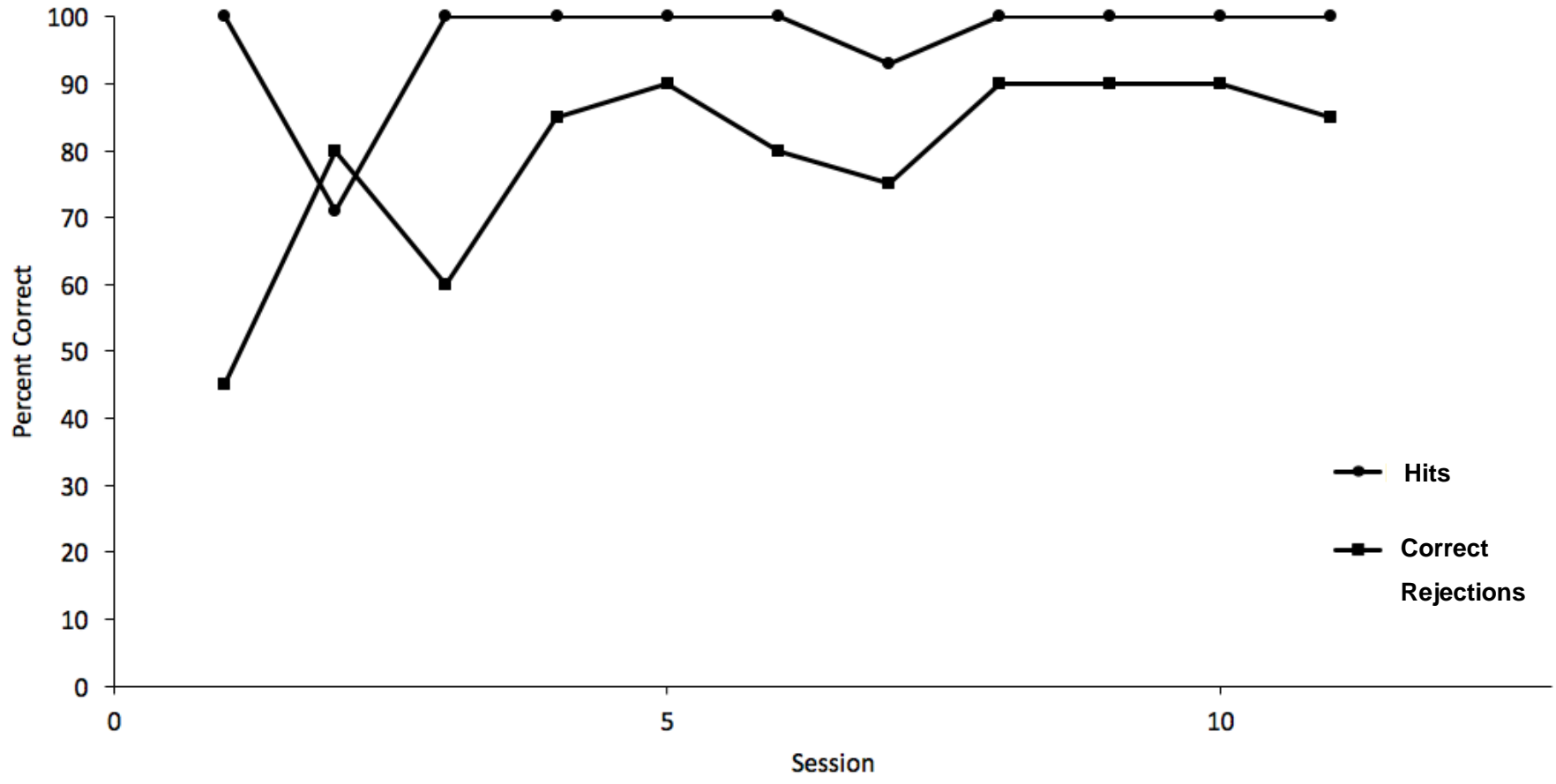


Figure 22. Hit rate plotted against the combined correct rejection accuracy (Luna).

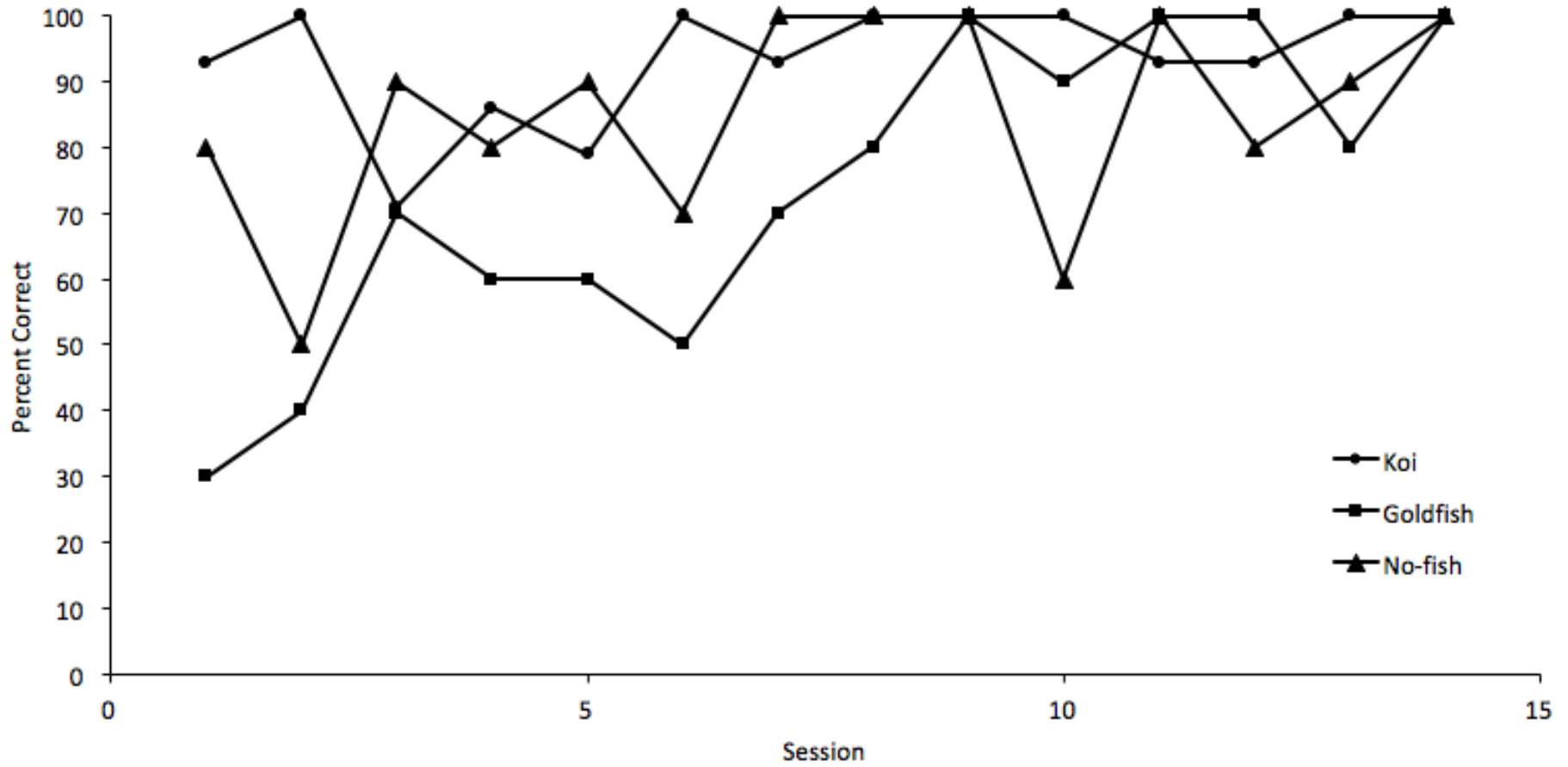


Figure 23. Mica's performance across three sample types (i.e., koi carp, goldfish, no-fish).

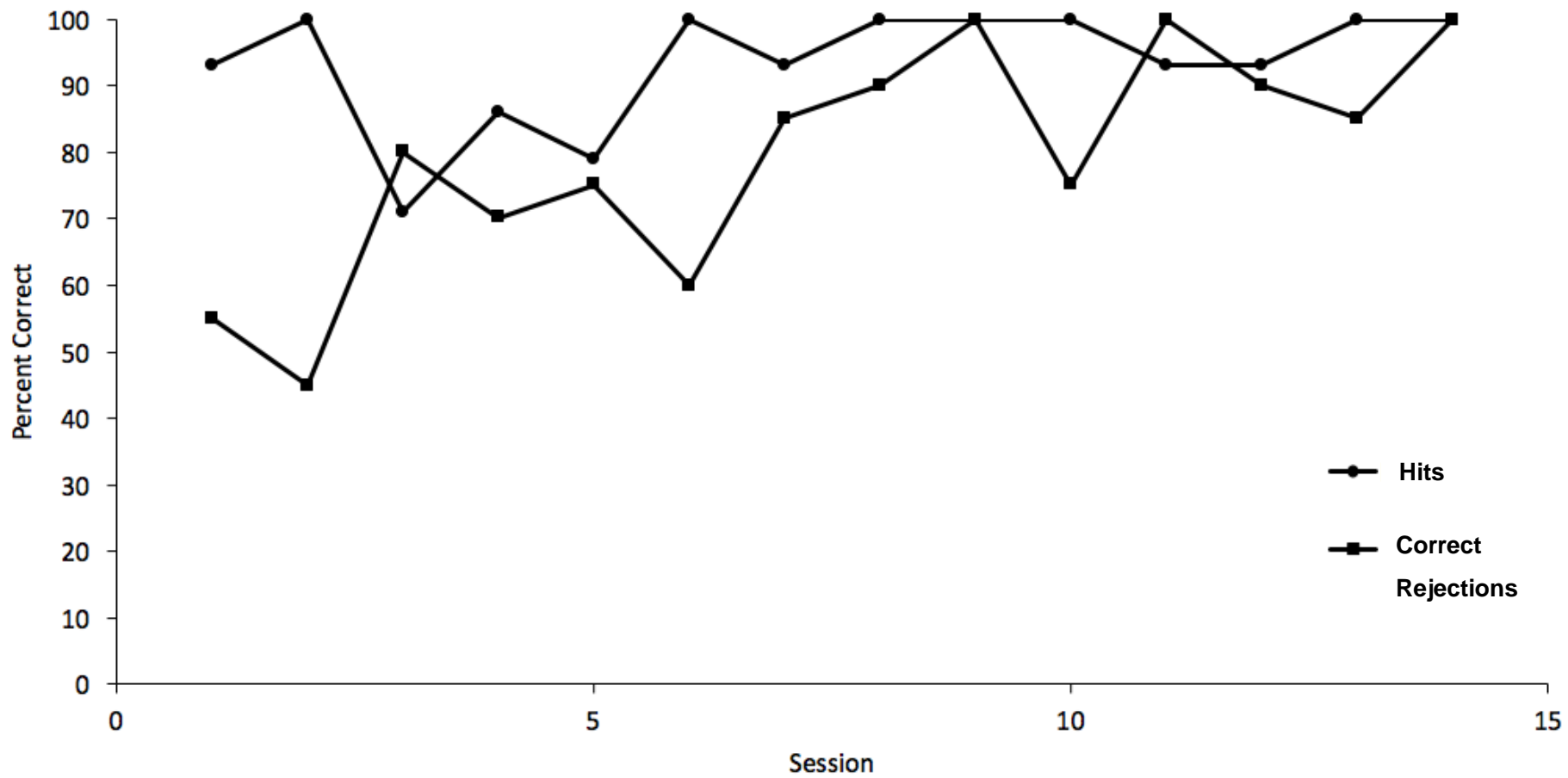


Figure 24. Hit rate plotted against the combined correct rejection accuracy (Mica).

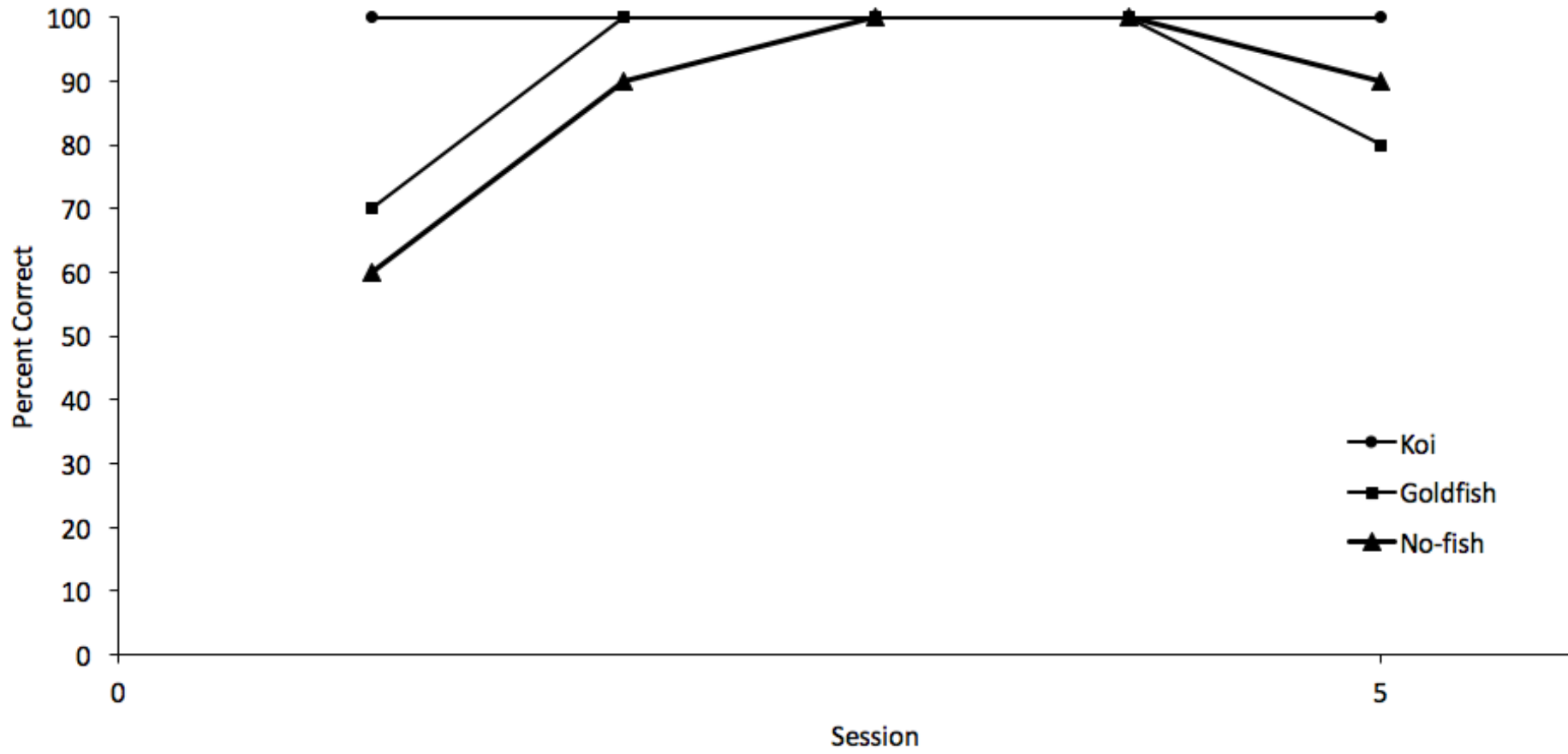


Figure 25. Ruby's performance across three sample types (i.e., koi carp, goldfish, no-fish).

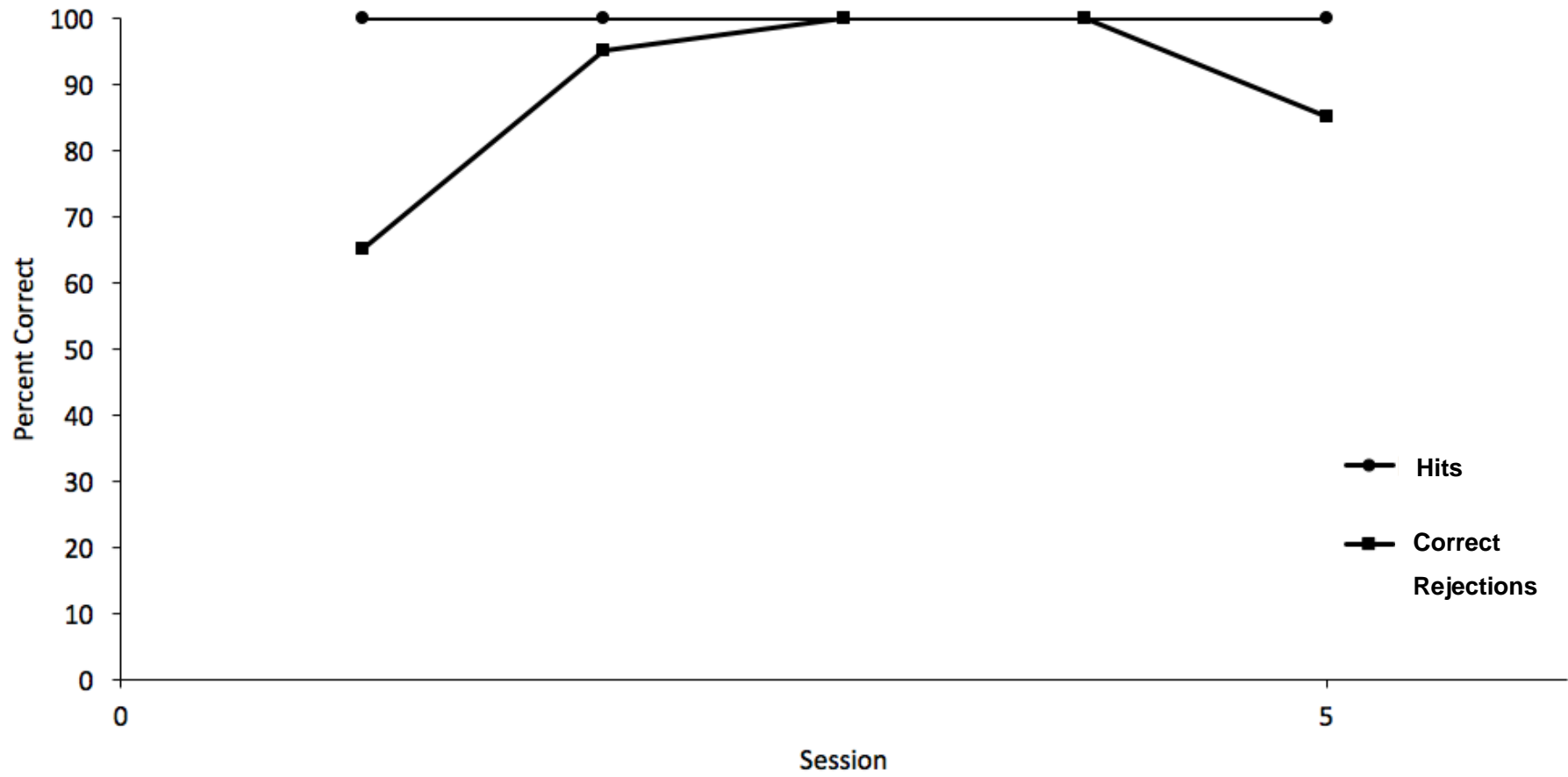


Figure 26. Hit rate plotted against the combined correct rejection accuracy (Ruby).

## 5.4 Discussion

All three dogs in this experiment successfully discriminated koi carp, goldfish, and no-fish samples. These are among the first published results to demonstrate dogs' ability to discriminate taxonomically-similar aquatic species.

In Experiment 3, all three dogs required either the same or additional sessions to achieve the termination criterion (i.e., Luna, 11; Mica, 21; Ruby, 11), as compared to the present experiment. From the outset it was assumed that this task would be more difficult than the previous task (i.e., that dogs would require longer to achieve the discrimination), as koi carp and goldfish are closely-related fish species that are both members of the Cyprinidae family. Catfish, on the other hand, are members of an entirely different family of fish (i.e., Ictaluridae). One possible explanation for this unexpected result is that prior to Experiment 3 the dogs had only been exposed to one species of fish, and that species of fish was always considered a positive sample. In Experiment 3, catfish were introduced as an additional negative sample, and the contingencies were arranged so that trials involving species other than koi carp would not produce a food reinforcer, regardless of the behaviour of the dogs. It is possible that this learning carried over into Experiment 4 and enabled dogs to respond at a higher accuracy and therefore complete the experiment within fewer sessions.

Two dogs (Luna and Mica) correctly rejected, on average, more no-fish samples than goldfish samples. This is interesting, given that the opposite effect was observed in the previous experiment with catfish and no-fish samples; however, the trend appears to be more pronounced in this experiment. The most

likely hypothesis for this is that dogs simply made more errors given the increased difficulty of this task.

# Chapter Six

## General discussion

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The results of the experiments described in the previous chapters provide compelling evidence for the utility of dogs in freshwater species detection. Not only has it been demonstrated that dogs are able to detect the presence of an invasive fish species (i.e., koi carp) from water samples, they are also able to discriminate between at least three species of fish (i.e., koi carp, catfish, and goldfish) at concentrations similar to those found within the natural environment. To the author's knowledge, this is the first published study of its kind to systematically examine the utility of dogs for aquatic species detection and one of the only studies that has provided evidence of dogs' ability to discriminate among taxonomically similar species (see Browne et al., 2015; and Lin et al., 2011). The results of this research have important implications for the management and conservation of waterways in New Zealand and around the world.

### 6.1 Olfactory detection in waterways

The use of detection dogs in applied settings is certainly no new phenomenon; for example, detection dogs have been used in New Zealand since the 1890s (Hill & Hill, 1987). However, this study details a unique and unprecedented application of detection dogs (i.e., as aquatic species detectors). According to Wilcox et al. (2013), there are three periods during which species detection efforts should be focussed: (1) after the invasive species is introduced, (2) once the invasive species has become well-established and native species are in decline, and (3) after

eradication attempts have been made to control the invasive species. Scent detection dogs could provide important information by learning to indicate the presence or absence of multiple species across all three phases of this continuum, and could be used to screen for particular species in areas where they are suspected to exist. The results of this study show that dogs are able to detect the presence of koi carp and can learn to largely ignore the presence of at least two other species of fish commonly found alongside koi carp, at an equivalent biomass density of approximately 37.4 kg/ha. Given their exceptionally high performance at the lowest dilution (i.e., 0.098%), it is possible that dogs may be able to detect fish at even lower biomass concentrations, allowing certain areas to be flagged for further investigation. For example, if koi carp are suspected to exist in a given area but are unable to be detected there by conventional methods (i.e., visual observation, netting, electrofishing), dogs may be able to detect their presence and thus spark a more thorough investigation (i.e., through an electrofishing expedition, or the application of genetic detection techniques such as eDNA).

The selection of koi carp as the target scent during this study was the first logical choice given their devastating impact on freshwater ecosystems (Collier & Grainger, 2015). However, it is important to note that dogs could potentially be trained to detect any target scent using the apparatus and procedures described in the previous chapters, including those of threatened native species. The strength of this procedure is that it can be adapted according to conservation priorities for any species, whether native or invasive.

## 6.2 Advantages over conventional detection methods

The limitations of conventional aquatic survey methods, and those associated with traditional canine scent detection, have been discussed in Chapter 1. For instance, conventional aquatic survey methods are often expensive (Daniel et al., 2009; Hicks et al., 2015a; Sigsgaard et al., 2015; Smart et al., 2016; Stoeckle et al., 2017), time-consuming (Sigsgaard et al., 2015; Stoeckle et al., 2017; Tyre et al., 2003; Wood et al., 2013), invasive (de Villiers, 2013; Snyder, 2003a, 2003b), and prone to error (Beukema & de Vos, 1974; Clark et al., 1991; Stoeckle et al., 2017; Symondson, 2002; Tyre et al., 2003; Wilcox et al., 2013). There are also a number of problems related to traditional canine scent detection tasks, including line-up arrangements and fieldwork (Chambers et al., 2015; Rebmann et al., 2000; Sargisson et al., 2010; Savidge et al., 2011), and those associated with influence of human subjectivity and cueing (Lit et al., 2011; Samhita & Gross, 2013; Zubedat et al., 2014). The methodology used in this study largely addresses these issues by proposing a system that is less time- and cost-intensive, less invasive, highly accurate, and relatively immune to human bias and influence.

Discussions regarding the adoption of new technologies often involve reservations about whether or not these methods actually provide added value, in terms of accuracy and/or cost-efficiency, over traditional procedures. The question of accuracy will not be answered adequately until further studies have been conducted using water samples taken during field surveys. However, the results of Experiments 1-4 in this study, and those reported in other applied scent detection research (e.g., insects, Brooks et al., 2003, Gsell et al., 2010, Hoyer-Tomiczek et al., 2016, Lin et al., 2011; rodents, Gsell et al., 2010; snakes, Savidge

et al., 2011; tortoises, Cablk & Heaton, 2006; bats, Chambers et al., 2015; gecko and tuatara, Browne et al., 2015; and forest carnivores, Long et al., 2007), suggest that this is likely not an issue.

This procedure provides a relatively fast and efficient solution for evaluating field samples without multiple dogs being required to visit each site of interest. A typical session involving 17 unique samples lasts approximately five minutes and allows for each sample to be evaluated twice by the same dog. If, for example, the standard is set so that at least four out of five dogs must indicate on a sample for it to be considered positive, the entire process for evaluating samples from up to 17 different locations would take just 25 minutes.

Some concerns might be raised over the investment of time, and therefore monetary expenses, required for training dogs for scent detection tasks. In this study, the initial training time varied among individual subjects, with some able to learn the task remarkably quickly. For example, one dog (Ruby), took just 30 minutes to reliably operate the apparatus and begin discriminating between positive and negative samples. Other dogs took several days, depending on how readily they habituated to the study location and whether or not the relevant establishing operations were in effect (i.e., whether the dog kibble used during the experiment functioned as a reinforcer). Others still were unable to pass the initial training phase. Indeed, most of the dogs recruited in this study were eventually withdrawn; a finding that is relatively common in canine detection work. Maejima et al. (2007), for instance, report that only 30% of detection dogs in Japan successfully pass training. In the present study, only 38% of dogs passed the initial

training, and only 23% passed all four experiments. Given these rather dismal figures, it is clear that the selection of appropriate dogs is crucial for successful scent detection work. Of the many behavioural characteristics of dogs, Maejima et al. (2007) identify two that are the most predictive of success during scent detection tasks: (1) desire for work; and (2) distractibility. Unfortunately, ordinary dog kibble (as would work in the food dispenser available to this study) is not an effective reinforcer for many dogs, nor do all dogs readily continue to work when exposed to noise, movement, and other environmental distractions. This makes the recruitment of appropriate dogs relatively difficult. However, the data from Experiment 1 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.

If the procedure described in this study is used as a tool for applied research and conservation management, costs can be further minimised by using pet dogs, rather than laboratory dogs, as was used in the current study. This avoids a number of additional expenses and ethical issues relating to the housing and care of laboratory animals. Costs might also be mitigated by using graduate students or volunteers to train the dogs and run experiments, and also collect samples from various locations in the field.

### **6.3 Limitations**

It was observed in some sessions that multiple dogs falsely indicated a sample as positive on the same trial during both rotations of the apparatus. Even though steps were taken to ensure sample fidelity, certain negative samples may have

become contaminated with the target scent during handling and preparation. However, if, on the rare occasion control samples did become contaminated, dogs were still indicating the presence of the target scent, which may support previous reports of dogs' outstanding olfactory sensitivity. Had samples remained uncontaminated, dogs' correct rejection rates might have been even greater than those reported in this study. Alternatively, it is also possible that dogs may have simply been "wrong" during these trials, and that samples did remain uncontaminated throughout the study, although this cannot be verified.

One systematic difference between samples in experiments 2-4 that was not fish-related was identified, and therefore may have impacted results. Small adhesive labels were placed on the outer edge of positive segments (i.e., vertically for koi carp, and horizontally for catfish and goldfish), and no labels were designated for segments containing control samples. This was done in order to improve efficiency as each segment was required to be removed, cleaned, and re-randomised between sessions. Although the labels were still technically inside the apparatus (i.e., below the apparatus lid), it is unlikely that they provided the dogs with any additional olfactory cues during these experiments.

#### **6.4 Future research**

Given that this is the first published research of its kind to investigate dogs' olfactory abilities using water samples in an automated apparatus, attempts were made to ensure that the experimental procedure used would provide insight into dogs' ability to detect fish in real-world scenarios. However, the results of these experiments may not generalise to field conditions until further investigations

have been conducted. The samples in this study contained only water taken from aquaria that had or had not contained fish. It is unclear what impact other organic compounds (e.g., fine sediments, plant materials, aquatic fauna) will have on detection accuracy when dogs are exposed to samples that contain numerous other non-target odours. Therefore, the next logical step would be to evaluate dogs' performance with water samples taken from areas in the field in which koi carp are known or known not to exist. Further research could also seek to discover lower-limit detection thresholds for koi carp given that the results of Experiment 2 suggest that performance may remain stable even at lower dilutions. A headspace analysis could be conducted on samples consistently indicated or incorrectly rejected by the dogs to see which compounds might be serving as discriminative stimuli. Dogs could also be exposed to hybrid species (e.g., koi carp-goldfish hybrids) as a control sample to discover whether dogs can be taught to ignore hybrids and respond exclusively in the presence of purebred fish. These are just a few of many areas where future research efforts might be focussed.

## **6.5 Conclusion**

The results of this study provide evidence that dogs can detect and discriminate at least three freshwater fish species from water samples at dilutions similar to those found within the natural environment. Dogs can detect koi carp at concentrations below the threshold at which habitat degradation begins to occur (Badiou & Goldsborough, 2010; Bajer et al., 2009), suggesting that dogs may become an important tool for conservation management in the years to come. Lodge et al. (2012), describing the promise of eDNA technology in an article titled

“Conservation in a cup of water”, writes “With eDNA, a lot can be learned from a cup of water” (p. 2557). Considering the results of this study, one might also assert: “With dogs, a lot can be learned from a cup of water”.

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# Appendix A

## **DOG BEHAVIOUR RESEARCH** *Initial Enquiry Form*



THE UNIVERSITY OF  
**WAIKATO**  
*Te Whare Wānanga o Waikato*

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

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

**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 new places?**

**Yes / No**

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

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

If **no**, please explain briefly: \_\_\_\_\_  
\_\_\_\_\_  
\_\_\_\_\_

**Does your dog like working for food?**

**Yes / No**

If **no**, please explain briefly: \_\_\_\_\_  
\_\_\_\_\_  
\_\_\_\_\_

**Can your dog eat any food, including kibble (biscuits) and different kinds of meat products?** **Yes / No**

If **no**, please explain briefly: \_\_\_\_\_  
\_\_\_\_\_  
\_\_\_\_\_

**Is your dog comfortable with people getting near their food?** **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'.  
(We will not necessarily have more than one dog at the training facility at once. If we do, it will be with permission of all owners and the dogs will be contained separately.)

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 Univeristy of Waikato main campus)?** **Yes / No**

Please indicate which times are more convenient: \_\_\_\_\_  
\_\_\_\_\_

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

**Please email this form to:**

# Appendix B

## **CONSENT FORM** *Participant's Copy*



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**

Jesse Quaife  
Dr Tim Edwards  
Dr Clare Browne

### **PURPOSE OF THE PROJECT**

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

### **DESCRIPTION OF PROCEDURE**

Water 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 water sample, and to indicate if the samples have/not contained certain fish species. Training will be achieved using food treats as positive reinforcement.

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

### **COSTS TO OWNER**

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

### **WITHDRAWING MY PET FROM THE PROJECT**

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

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

### **ADDITIONALLY**

I understand that participation in this project involves a commitment to bring my pet to the dog facility according to a schedule realised in cooperation with the researchers. Upon completion of the research, I will have access to my dog's data and the general findings from the research project.

### **AUTHORISATION**

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

I give consent for my dog to be at the research facility in the presence of other dogs: **Yes / No**

My dog is friendly towards other dogs: **Yes / No**

I give consent for videos of my dog to be shown for other purposes (presentations, lectures, etc.): **Yes / No**

Pet's name: \_\_\_\_\_

Owner's name: \_\_\_\_\_

Owner's signature: \_\_\_\_\_ Date: \_\_\_\_\_

Researcher's signature: \_\_\_\_\_ Date: \_\_\_\_\_

# Appendix C

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## Standard Operating Procedure: Water Sample Collection and Preparation

### Guidelines for Water Sample Collection and Preparation

#### 1. Purpose

This standard operating procedure (SOP) provides guidelines and standardised procedures to be adopted during collection and preparation of water samples that have been collected from the Aquatic Research Centre, located at FC2-G on the University of Waikato campus. Only those with prior induction training are authorised to enter this facility and collect samples.

#### 2. Draining tanks in preparation for sample collection

- 2.1. Covered shoes must be worn on the premises at all times.
- 2.2. Place all personal belongings in the office (Room G.01 on facility map).
- 2.3. Put on a white lab coat and a pair of disposable gloves.
- 2.4. If water samples are to be collected the next day, the correct tank will need to be drained, cleaned, and refilled 24 hours prior to sample collection. If today is Wednesday, the correct tank is "Tank 2". If today is Thursday, the correct tank is "Tank 3".
- 2.5. Remove the tank cover and plastic plug pipe from the tank.
- 2.6. Turn the water off by adjusting the blue lever situated on the wall above the tank so that it is sitting at a horizontal angle.
- 2.7. Using the green scratchy pad provided, carefully scrub the inside of the tank as it is draining, taking care not to touch the fish.
- 2.8. Wash any debris down the drain with the hose inside the tank (the pipe can also be used to swish any obvious debris towards the hole).
- 2.9. Once the tank has been scrubbed clean and is free of obvious debris, replace the plastic plug pipe and fill the tank to the line marked "2" on the pipe.
- 2.10. Turn off the water and replace the tank cover, making sure the oxygen hose is still below the water's surface.
- 2.11. Remove the lab coat and dispose of the gloves.
- 2.12. Lock the door on exiting.

#### 3. Collection of water samples

- 3.1. Covered shoes must be worn on the premises at all times.
- 3.2. Place all personal belongings in the office (Room G.01 on facility map).
- 3.3. Put on a white lab coat and a pair of disposable gloves.

## Standard Operating Procedure: Water Sample Collection and Preparation

- 3.4. Using the negative sample beaker provided (indicated by “=”), collect a sample of water from the negative sample tank (the tank that is closest to the wall that contains no fish). Take care not to submerge gloves in the sample or tank water.
- 3.5. Pour the water from the beaker into the negative specimen bottle (indicated by “=”). Repeat until the bottle is full and attach the lid.
- 3.6. Wipe the outside of the specimen bottle with a disposable paper towel.
- 3.7. Place the negative specimen bottle into the metal bottle carrier.
- 3.8. Repeat steps 3.4-3.7 with the other negative specimen bottle.
- 3.9. Clean the negative sample beaker with hot soapy water and rinse thoroughly.
- 3.10. Dry the beaker and place it on the left side of the grey plastic storage box located on the floor under the negative tank.
- 3.11. Check to see which positive sample tank is to be collected from. To do this, carefully remove the water hose from each tank to check which is flowing. The correct tank is indicated by the absence of running water, which will have been turned off 24 hours prior to sample collection to ensure potent samples. This tank will also be half-full. On Thursdays the collection tank is Tank 2, and on Fridays it is Tank 3.
- 3.12. Carefully remove the cover from the positive sample tank and collect a sample from this tank by repeating steps 3.4-3.6 using the positive sample beaker (indicated by “+”). Take care not to agitate the fish when sampling from the positive sample tank. Do this by minimising noise and movement, and dipping the beaker into the water at a distance from the fish.
- 3.13. Place the full positive sample bottle in personal backpack.
- 3.14. Carefully replace the tank cover and turn the water back on by adjusting the blue lever so that it sits on a slight angle. The water should be flowing (not gushing) at a steady rate.
- 3.15. If samples are to be collected the next day, follow the steps in 2.4-2.10 above.
- 3.16. Wash the positive sample beaker with hot soapy water.
- 3.17. Dry the beaker and place it on the right side of the grey plastic storage box that is located on the floor under the negative tank.
- 3.18. Remove the lab coat, and dispose of the gloves.
- 3.19. Lock the door on exiting.
- 3.20. Store the collected water samples at approximately 4°C for a maximum of 24 hours.

## Standard Operating Procedure: Water Sample Collection and Preparation

### **4. Preparation of water samples for dilution**

Samples are diluted at the dog facility located at TTH1 on the University of Waikato campus. Only those with prior training are authorised to dilute samples.

- 4.1. Place 10 clean sample jars on the right side of the preparation table, and seven on the left (separated).
- 4.2. Place a white label horizontally across the front of each of the 10 jars on the right to indicate that these are the negative samples.
- 4.3. Place a white label vertically across the front of each of the seven jars on the left to indicate that these are the positive samples.
- 4.4. Using the 100 ml plastic syringe provided in the bag labelled "=", fill each of the 10 jars on the right with 100 ml of water from the negative sample bottle.
- 4.5. Place each of these 10 jars in the apparatus according to predetermined set up.
- 4.6. Using this same syringe and bottle, fill each of the seven positive sample jars on the left with water according to the required dilution. For example, if using a dilution of 25%, fill each jar with 75 ml of water from the negative sample bottle.
- 4.7. Once the appropriate amount of negative sample water has been placed in each jar, use the appropriate plastic syringe (or pipette attachment) provided in the bag labelled "+" to fill each of these seven positive sample jars with water from the positive sample bottle until the desired dilution/volume is reached. For example, if using a dilution of 25%, fill each jar with 25 ml of water from the positive sample bottle, so that the total volume of each positive sample jar is 100 ml (same as the negative sample jars).
- 4.8. If using smaller syringes (i.e., 10 ml) or auto-pipettes that do not fit into the sample bottle, fill a spare sample jar with water from the positive bottle and use the appropriate syringe/auto-pipette to transfer this water into the other jars.
- 4.9. Place each of these seven positive sample jars in the apparatus according to the predetermined set up.

### **5. Transportation of sample bottles**

- 5.1. Both negative sample bottles are transported in the metal bottle carrier.
- 5.2. The positive sample bottle is transported separately inside a personal backpack.

## Standard Operating Procedure: Water Sample Collection and Preparation

### **6. Cleaning procedures for storage bottles and preparation table**

- 6.1. Sample bottles are cleaned with hot soapy water and rinsed thoroughly at the end of every collection day. Similar to the other procedures, the negative sample bottle is always handled first.
- 6.2. If any spillage of sample water occurs on the preparation table, this is cleaned with the alcohol solution and disposable paper towels. If the spillage contained positive sample water, gloves must be changed before handling any negative samples.

### **7. Storage of plastic syringes/pipette attachments**

- 7.1. Plastic syringes and pipette attachments that are used for the negative samples are stored in the plastic snap-lock bag labelled "=", and those that are used for the positive samples are stored in the plastic snap-lock bag labelled "+". These bags are placed separately (not touching) inside the plastic divider tray that is stored inside the left cupboard of the preparation table. If there is water on the outside of these bags, they must be dried with a disposable paper towel before storing them inside the plastic divider. Snap-lock bags should be replaced periodically.