Locating Whitebait (*Galaxias argenteus*) Eggs Via Canine Scent Detection

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Abstract

The New Zealand whitebait fishery consists of five species of Galaxiidae fish and constitutes a culturally important commercial and recreational resource for New Zealand. Despite efforts by the Department of Conservation and regional councils, there has been a significant decline in whitebait over the past several decades, with three of the five whitebait species (īnanga, giant kōkopu, kōaro) now classified as 'declining' and one (shortjaw kōkopu) considered 'threatened'. Adult fish spawn on riparian vegetation near river mouths during spring tides, the eggs then develop aerially until the following spring tide when they hatch, and the larvae disperse into coastal estuaries. Anthropogenic activities such as flood management, vegetation removal and reduced water quality have led to widespread loss of suitable spawning habitat. Identification of spawning habitat is a key aspect to conserving whitebait species. However, visual surveys for spawning sites are time consuming and spawning areas difficult to predict, as most species do not return to the same spawning site each year. Scent-detection dogs may provide an efficient and effective way of locating whitebait nests, allowing increased protection of spawning areas against disturbance. Four dogs were trained to reliably detect and discriminate giant kōkopu (*Galaxias argenteus*) eggs from garden snail (*Cantareus aspersus*) eggs, grass, and blank (no scent) samples within a laboratory-based line-up with a high level of accuracy (≥90% hit rate and ≤10% false alarm rate). Progressing from this, one dog worked on scent line-ups outdoors and demonstrated the ability to reliably detect giant kōkopu eggs (≥90% hit rate and ≤10% false alarm rate), with increasing environmental complexity, as a progression towards application of these dogs for whitebait egg detection in the field. This research provides an opportunity to further explore the use of dogs to detect whitebait spawning sites in the field, potentially providing a new tool for whitebait conservation in New Zealand.

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Chapter 1

Introduction

'Whitebait' is a collective term given to small juvenile fish that are caught in large numbers for consumption commercially or recreationally (Wylie et al., 2016; Yungnickel et al., 2020). Worldwide, whitebait consists of a variety of different species; however, in New Zealand, whitebait catches usually consist of the juvenile form of five Galaxiidae species which are caught as they move from coastal waters into freshwater catchments (Charteris & Ritchie, 2002; Yungnickel et al., 2020). The practice of 'whitebaiting' has high cultural significance in New Zealand, having been an important traditional food source for Māori (Fyfe & Bradshaw, 2020), and still practiced in the modern day recreationally. Whitebaiting is seen as a way of getting into nature, fostering cultural foodways, and provides a strong sense of identity within both Māori and non-Māori communities (Haggerty, 2007). However, within the last few decades there has been a noticeable reduction in whitebait catches in New Zealand due to stressors such as habitat destruction and declining water quality (Yungnickel et al., 2020). As a result, three of the five Galaxiidae species caught as whitebait are now considered 'declining' and one is considered 'threatened' (Department of Conservation, 2021*b*).

1.1 Species in New Zealand

The Galaxiidae family are the most widely distributed freshwater fish in the southern hemisphere (McDowall, 1964). Most Galaxiidae are found in New Zealand and Australia, but they are also found in South Africa, Chile, Argentina, New Caledonia, Lord Howe Island, Tasmania, the Falkland Islands and the Chatham, Auckland, and Campbell Islands (Allibone, 2003; McDowall, 1964; McDowall, 2006*a*). In New Zealand, there are 26 Galaxiid species, of these, five are found in the whitebait catch. These five species are the only New Zealand galaxiid species that are known to be diadromous (i.e., migrates between marine and freshwater environments) and consist of: īnanga (*Galaxias maculatus*), kōaro (*G. brevipinnis*), banded kōkopu (*G. fasciatus*), giant kōkopu (*G. argenteus*) and shortjaw kōkopu (*G. postvectis*) (McDowall 1964; Yungnickel *et al*. 2020).

Īnanga (*Galaxias maculatus*)

Īnanga are the most common species found within the whitebait catch (Yungnickel *et al*., 2020). They are small, slender fish with adults typically reaching 40-120 mm in length (Plew *et al.,* 2007). Īnanga possess poor climbing ability and are hindered by in-stream obstacles when attempting to migrate back upstream from their coastal habitats (Baker & Boubée, 2006). They inhabit a diverse range of gently flowing or still lowland wetlands, streams and rivers and usually only have a life span of around 1-year (Baker, 2003; McDowall, 1990; Plew *et al*., 2007). They differ from the other Galaxiid whitebait species by being 'marginally' catadromous by migrating down to estuaries to spawn, where they lay their eggs amongst intertidal vegetation during spring tides (Baker, 2006). Īnanga have a 'declining' conservation status, with a total area of occupancy being >10,000 ha and a predicted decline of 10-70% (Dunn *et al*., 2018). Īnanga are distributed throughout rivers in every region of New Zealand, however, are found in significantly lower proportions in Buller than in Waikato, Canterbury and Southland (Yungnickel *et al*., 2020).

Kōaro (*G. brevipinnis*)

Kōaro have a streamlined body shape with large pectoral fins which give them the ability to climb vertical surfaces like waterfalls and assist them when moving in fast flowing, turbulent water (McDowall, 1990; O'Connor & Koehn, 1998). They are selective in habitat, typically living in forested upland cold, fast flowing streams, penetrating much further inland to higher altitudes through their climbing ability (Baker & Hicks, 2003; McEwan & Joy, 2014). Kōaro reach sexual maturity at c. 2 years of age and survive spawning much longer, having been documented to live up to 15 years, typically growing to 160-180 mm in length (Baker & Hicks, 2003). Kōaro have a 'declining' conservation status, with a total area of occupancy being >10,000 ha and a predicted decline of 10-70% (Dunn *et al*., 2018). Kōaro have been sampled in rivers from all regions apart from Auckland and Coromandel and have noticeably lower proportions within some rivers in Waikato, Bay of Plenty, Hawke's Bay and Canterbury (Yungnickel *et al*., 2020).

Banded kōkopu (*G. fasciatus*)

Banded kōkopu are adept climbers, allowing them to migrate further inland where they typically inhabit small, forested tributaries (West *et al*., 2005). Males reach sexual maturity from c. 2 years of age and females c. 4 years, living up to at least 9 years and reach lengths of 170-180 mm at adulthood (West *et al*., 2005). Their diet largely consists

of terrestrial food falling from bankside vegetation and overhanging trees, explaining their preference for forested streams (West *et al*., 2005). Banded kōkopu are considered 'not threatened', having large stable populations throughout New Zealand (Dunn *et al*., 2018). Banded kōkopu have been found in all regions but at noticeably lower proportions in rivers within Canterbury, Otago, and Southland (Yungnickel *et al*., 2020).

Giant kōkopu (*G. argenteus*)

Giant kōkopu are the largest fish in the galaxiid family, reaching to 300-400 mm in length and are long-lived, having been found to live for over 20 years (Bonnett & Skyes, 2002; McDowall, 1990). They are generally found at lower elevations in wetlands, lakes, rivers, and streams that have deep, gently flowing water with a lot of instream and bankside cover (Bonnett & Skyes, 2002). Giant kōkopu are one of the only species of whitebait to be commercially farmed in an emerging aquaculture industry, as their size and high fertility makes them more suitable for a commercial environment compared to smaller species like īnanga (Lulijwa *et al*., 2021; Wylie *et al*., 2016). Giant kōkopu have a 'declining' conservation status, with an estimated population of 20,000-100,000 adult individuals nationally and a predicted decline of 10-50% (Dunn *et al*., 2018). Giant kōkopu are most commonly found in rivers along the west coast of both islands and around the Cook Strait (Tasman-Nelson, Wellington regions) (Yungnickel *et al*., 2020).

Shortjaw kōkopu (*G. postvectis*)

Shortjaw kōkopu are an uncommon species that can be found sporadically in cold, fast-flowing streams in forested areas, where they hide under boulders (McDowall *et al*., 1996; McEwan & Joy, 2014). They are usually found in small numbers and are considered the most threatened of New Zealand's galaxiid species, however this may be due to sampling methods not reaching their under-boulder habitats (Allibone *et al*., 2003; McDowall *et al*., 2006). They typically grow to approximately 150-200 mm and live for around 15 years (Allibone *et al*., 2003). Shortjaw kōkopu have a 'nationally vulnerable' conservation status, with only an estimated 5,000-20,000 adult individuals nationally and a predicted decline of 10-50% (Dunn *et al*., 2018). Due to the conservation status of shortjaw kōkopu, population distribution is difficult to establish, but individuals have been found within sampled rivers in Manawatū, Bay of Plenty, Buller, and the west coast of both islands (Allibone *et al*., 2003; Bowie and Henderson, 2002; Yungnickel *et al*., 2020).

1.2 Whitebait life cycle

All five whitebait species are amphidromous, they migrate bi-directionally between marine and freshwater environments, laying their eggs in freshwater, where the larvae hatch and migrate to sea for several months and then return upstream where they grow to sexual maturation and reproduce (McDowall, 2010). Whitebait typically lay their eggs supratidally in riparian vegetation along river mouths and estuaries during spring tides. The embryos develop for 2-4 weeks before hatching when inundated by the next spring tide and are swept out to sea or to lakes (Franklin *et al*., 2015; Stevens *et al*., 2016). Once hatched, the larvae develop for 4-6 months in the pelagic zone in coastal areas, before returning to rivers in large mixed species shoals (Hickford & Schiel, 2011*a*). It is during this mass return to rivers that whitebait are caught as the maturing larvae swim upstream. Those that are not harvested in the whitebait catch continue upriver where they continue to grow, before reaching sexual maturity and moving to spawning sites (Franklin *et al*., 2015; Hickford & Schiel, 2014).

New Zealand's whitebait species are widely distributed throughout the country (Figure 1.1). Typically, higher proportions (c. 85%) of īnanga are found in catches nationally, with the other four species occurring more variably at lower proportions (c. 0-7%) (Yungnickel *et al*., 2020). High proportions of kōaro, banded and giant kōkopu have been found on the West Coast, Southland, Waikato, Bay of Plenty, Manawatu-Wanganui, Wellington and Tasman-Nelson (Yungnickel *et al*., 2020). Typically, east coast regions of New Zealand, particularly Canterbury, see lower proportions of non-īnanga whitebait species than west coast regions (Yungnickel *et al*., 2020). Species composition differs in each region depending on the time of year and river type. Kōaro are more often found in larger river systems, whereas banded kōkopu prefer smaller, high forest cover catchments, river selection by these two species is thought to be encouraged by attraction to odours released by adult conspecifics (Baker & Hicks, 2003; Yungnickel *et al*., 2020). Temperature is also believed to affect which rivers whitebait migrate to, with kōaro typically found in cold, glacier/mountain fed rivers and giant, banded, and shortjaw kōkopu migrating more frequently to warmer, forest covered rivers (Yungnickel *et al*., 2020). Īnanga are typically more general in their habitat choice and more tolerant to changes in water quality, which likely contributes to their abundance in sampling and catches nationwide (McDowall, 1990; Yungnickel *et al.*, 2020).

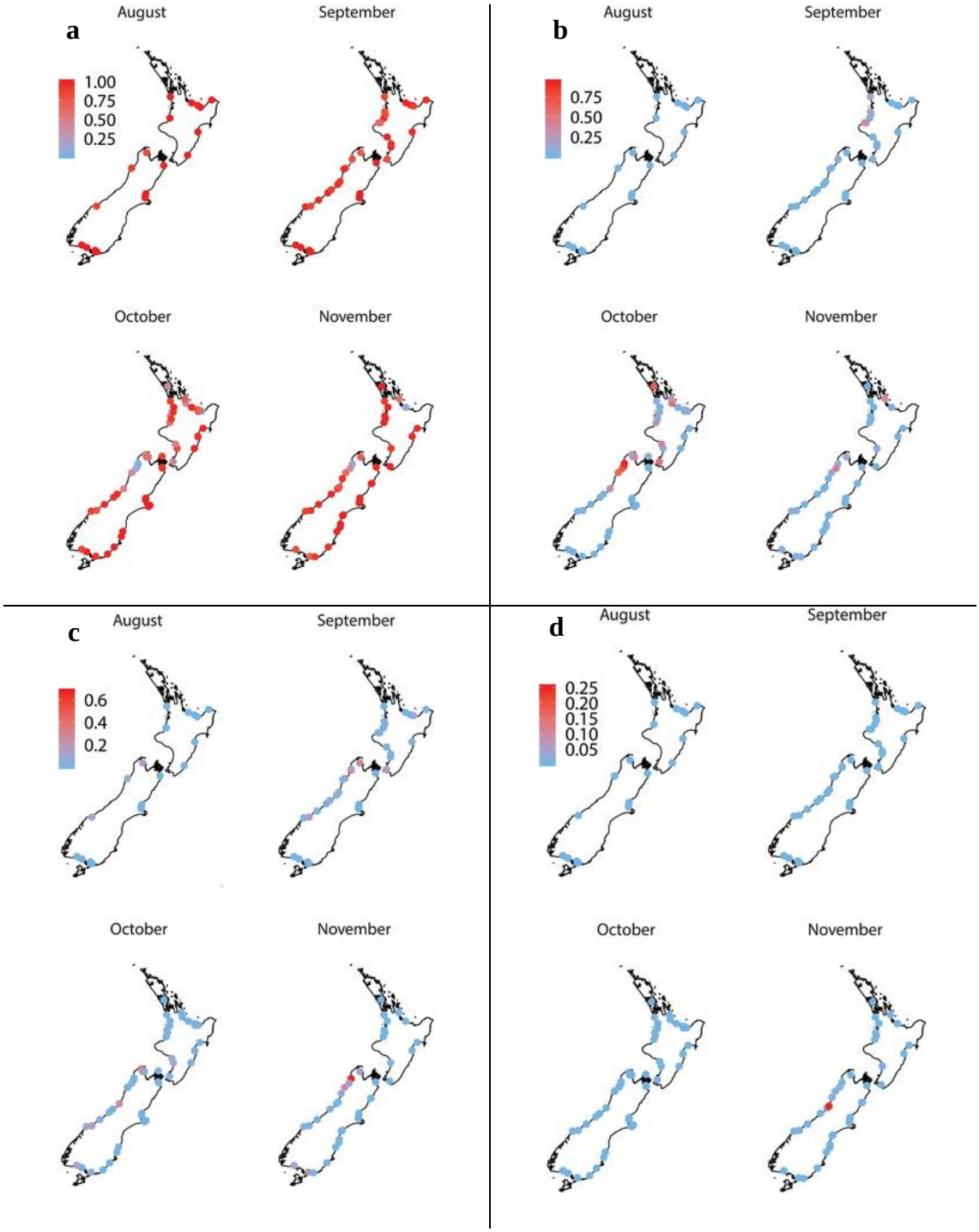

Figure 1.1 Proportion of (a) īnanga, (b) banded kōkopu, (c) kōaro, and (d) giant kōkopu in whitebait samples ($n \geqslant 100$ fish) collected over four months from 87 rivers around New Zealand. (Source: Yungnickel *et al*., 2020)

1.3 Factors effecting whitebait populations

It is suspected that whitebait numbers are in decline through reduced catches, but specific statistics on whitebait numbers have not been kept until the latter half of the 20th century (Hickford & Schiel, 2011*b*), with one of the first widespread studies occurring in 1965

(McDowall, 1965), but it had limited spatial and temporal coverage. Dedicated reviews (e.g., Allibone *et al*., 2010; Goodman *et al*., 2014) confirmed that adult whitebait numbers are declining, and three species are now classified as 'declining' and one is considered 'threatened' by the Department of Conservation (DOC). This decline has been attributed to anthropogenic activities such as wetland drainage, flood protection, and agricultural intensification that result in habitat degradation and a reduction in water quality (Hickford & Schiel, 2011*b;* Yungnickel *et al.,* 2020).

The introduction of dams, culverts and weirs have been essential in human development to control floods, generate electricity and to provide water for civil and irrigation purposes (Doehring *et al*., 2012; Jellyman & Harding, 2012). However, their introduction disrupts water flow regimes, severs migratory routes, and severely affects the biological, chemical, and physical processes that operate within freshwater ecosystems (Jellyman & Harding, 2012; Young *et al*., 2004). Whitebait species such kōaro and banded kōkopu prefer elevated stream reaches which are cooler and better oxygenated; and while both species are able to climb vertical structures, there are limits to this ability (Doehring *et al*., 2012). Dams and other disconnecting structures can sever connections to elevated environments, taking away access to preferred habitat for these species (Doehring *et al*., 2012). Īnanga, giant kōkopu and shortjaw kōkopu prefer warmer lowland waters, but habitat disconnection and loss has occurred in such waterways through flood control systems, eliminated flood inundation zones, loss of wetlands and the installation of weir gates and pumping stations (Doehring *et al*., 2012; Jowett, 2002). This restricts habitat available for whitebait, creating source and sink populations which lead to population bottlenecks and demographic deficits within whitebait populations (Hickford & Schiel, 2011*a*). Introduction of manmade structures like culverts can result in significantly increased water velocities downstream (Doehring *et al*., 2011). Increased velocity is thought to affect feeding capabilities of whitebait species who require lower velocities to feed on drifting invertebrates and other food sources adequately (Jowett, 2002; McDowall *et al*., 1996).

Deforestation, drainage of wetlands and agricultural development have caused changes in streams and rivers, resulting in large habitat reductions for whitebait species which impacted their distribution and abundance (Swales & West, 1991; Yungnickel *et al*., 2020). Sediment erosion into surrounding waterways has been shown to negatively affect water quality by increasing turbidity (Richardson *et al*., 2001). Increased turbidity reduces fish abundance by affecting the migration of fish species, which is particularly concerning

for whitebait species that undertake large-scale migrations to and from the sea, and are known to demonstrate avoidance behaviour of turbid waterways (Richardson *et al*., 2001; Rowe *et al*., 2000). Increased levels of nutrients from fertilisers are known to push freshwater systems into eutrophic states that impact in-stream flora and invertebrate abundance by decreasing species richness, affecting habitat and food availability for galaxiids and other fish species through the loss of fauna (McDowall, 2006*b*; Riis & Sand-Jensen, 2001; Stuart-Smith, 2006). Introduced toxicants into waterways from human activity also interferes with the ability of fish to maintain ion homeostasis and can result in increased uptake of metals, plastics, chemicals, and other toxicants, especially when respiratory requirements are increased during the energetically demanding migratory periods that are undertaken by galaxiids (Harley & Glover, 2014). Toxicants can accumulate within tissues, affect biological functions, and compete with essential ions such as sodium within the body of fish, leading to increased mortality in galaxiid species (Harley & Glover, 2014; McRae *et al*., 2019). For example, cadmium which is found in batteries and agricultural fertiliser, has been shown to cause oxidative stress in īnanga (McRae *et al*., 2019) and impair olfactory function in banded kōkopu (Baker & Montgomery, 2001).

The riparian zone along riverbanks is the boundary between terrestrial and aquatic environments and is vital in protecting streams from agricultural contaminants by filtering surface runoff, encouraging nutrient uptake and denitrification by flora (Hickford & Schiel, 2014; Quinn *et al*., 2001). Riparian zones are responsible for maintaining key aspects of instream habitat by providing adequate shading, stabilising the streambank, and providing essential habitat for whitebait to spawn (Hickford & Schiel, 2014; Quinn *et al*., 2001). Alterations to these zones by grazing livestock, urbanization, land clearing, coastal developments and flood control methods has resulted in increased water flows, river channelisation, sediment deposition, removal of vegetation, as well as changes to light, temperature, and humidity within riparian environments (Hickford & Schiel, *2011a;* Hickford & Schiel, 2011*b*). The success of spawning in whitebait species is closely correlated with the characteristics of their riparian spawning habitat, with preference given to *Schedonorus phoenix*, *Agrostis stolonifera* and *Juncus edgariae* grasses that provide the right height and density to influence temperature and humidity at ground level, as well as protect eggs from overexposure to ultraviolet light (Hickford & Schiel, 2011*a*; Hickford & Schiel, 2011*b*). Previous studies have found that when the riparian

environment is altered due to human activities, the success of īnanga spawning can be significantly reduced. (Hickford & Schiel, 2011*a*).

Introduced species can have a negative effect on whitebait populations through predation, competition, and habitat occupation (Stuart-Smith *et al*., 2007). Fish species such as brown trout (*Salmo trutta*), rainbow trout (*Oncorhynchus mykiss*) and other introduced salmonoid species have been shown to drive source-sink population dynamics, as well as cause an increase in avoidance behaviour by galaxiids, sometimes resulting in microhabitat segregations within streams (Woodford & McIntosh, 2010). There is also increased competition for food and habitat, with galaxiids being particularly vulnerable to predation by introduced salmonids and percid species (McDowall, 2007; Sowersby *et al*., 2016; Woodford & McIntosh, 2010). Predation of whitebait eggs by terrestrial and intertidal species such as field mice (*Mus musculus*), mud crabs (*Scylla serrata*) and opportunistic carnivorous arthropods has also been reported (Allibone, 2003; Baker, 2006).

Conservation efforts undertaken to help restore whitebait populations include monitoring by regional councils and regulations imposed by DOC that restrict recreational fishing of whitebait to certain times of the year, netting regulations, and restoration of riparian spawning zones. However, there is little knowledge of whitebait spawning sites, with sites usually being found at the interface of salt and freshwater in rivers and within the vegetation where elevated spring tide water levels reach (Hickford & Schiel, 2011*a*; Orchard & Schiel, 2021). Current methods to identify spawning sites are labour intensive, involving manual investigation of vegetation at survey sites and thorough inspection of root mats and stems (Orchard and Schiel, 2021). When eggs are found, there is an additional challenge of correct identification, as whitebait eggs can be morphologically similar to those of non-target species (e.g., slugs and snails) and DNA analysis on found eggs is often required to confirm correct identification of species (Franklin *et al*., 2015). A tool that can provide quick and accurate identification of whitebait nests would provide vital information on spawning habitats of whitebait species and would allow for better egg and habitat protection. Conservation scent dogs are one such tool that have potential in this setting as they have been found to be highly effective, easily acquired and relatively cheap to employ with a potentially higher success rate than using humans alone (DeMatteo, 2019).

1.4 Domestic dogs as scent detection instruments

The use of dogs (*Canis familiaris*) by humans as scent detectors dates back 12,000 years when they were used for hunting (Furton & Myers, 2001). Dogs place a comparatively large importance on smell to help them navigate the world around them, both functionally (e.g., locating food) and behaviourally (e.g., locating potential mates) with a highly attuned olfactory system that is approximately 10,000-100,000 times more sensitive than humans' (Craven *et al*., 2010; Kavoi & Jameela, 2011; Walker *et al*., 2006). Dogs have two components to their olfactory system: the main olfactory epithelium which is in the mucosa of the caudo-dorsal region of the nasal cavity, and the vomeronasal organ which is located between the oral and nasal cavity near the vomer bone close to the roof of the mouth (Kokocińska-Kusiak *et al*., 2021).When inhaling, air is split into two separate pathways within the nostril, with c. 12% of inhaled air being sent on the upper flow path to the olfactory region and the rest of the air sent on the lower path down the pharynx and into the lungs (Craven *et al*., 2010; Kokocińska-Kusiak *et al*., 2021). Odour molecules in the air sent to the upper flow pathway are deposited and accumulated, while air sent on the lower pathway is expired from the same pathway, prolonging exposure and accumulation of scent molecules to chemoreceptors on the olfactory epithelium (Craven *et al*., 2010; Kokocińska-Kusiak *et al*., 2021). The nasal cavity of dogs allows for odour molecules to be sampled separately in each nostril, permitting bilateral comparison of odour intensity and assisting with directional localisation of the odour source (Craven *et al*., 2010; Kokocińska-Kusiak *et al*., 2021). Canines have the capacity to detect an extensive variety of different odourants, with around 200 million olfactory receptor neurons and around 800 active olfactory receptor genes, that allows for odourant receptivity that is three orders of magnitude more sensitive than many of the instruments available today, at a fraction of the cost (Angle *et al*., 2016; Hayes *et al*., 2018; Quignon *et al*., 2012).

1.5 Current applications of scent-detection dogs

Dogs have been proven to be able to detect up to 10 different target odours in a fixed search scenario and have shown to learn additional odours with ease once the first odour is trained (Cablk & Heaton, 2006; William & Johnston, 2002). Odours are typically made up of a combination of different volatile organic compounds (VOCs) that are continually released into the environment (Leitch *et al*., 2013). VOCs have high vapour pressures and evaporate into the into the air when certain conditions are met, with different VOCs

becoming volatile at varying temperatures and humidity (Edwards *et al*., 2017; Hanks & Louglin, 2011). Dogs have demonstrated scent detection thresholds of an odour in ranges of parts-per-million (ppm) (e.g., 2,4-dinitrotoluene) to parts-per-trillion (ppt) (e.g., *n*-amyl acetate) (Craven *et al*., 2010; Leitch *et al*., 2013; Lorenzo *et al*., 2003). Hall *et al*. (2016) found that dogs are able to generalize compounds that have the same and similar carbon-chain lengths. This generalisation is thought to occur because structurally similar compounds activate overlapping olfactory glomeruli, eliciting a generalized behavioural response (Moser *et al*., 2019). Because of this generalisation, the need to train a response towards variations of the target odour is diminished (Moser *et al.,* 2019). Dogs have been trained to detect VOCs from explosives (Furton & Myers, 2001), drugs (Jezierski *et al*., 2014), human remains (Oesterhelweg *et al*., 2008), missing people (Lit & Crawford, 2006), oestrus in cows (Fischer-Tenhagen *et al*., 2011), cancers including colorectal (Sonoda *et al*., 2011), lung (McCulloch *et al*., 2006), prostate (Cornu *et al*., 2011), and cervical (Guerrero-Flores *et al*., 2017), as well as plant and animal products (Helton, 2009; Wasser *et al*., 2004; Moser *et al*., 2020). This versatility in scent detection targets has made them commonplace within sectors such as law enforcement, biosecurity in airports and ports, military service, medicine, and conservation (Browne *et al*., 2006; Furton & Myers, 2001; Johnen *et al*., 2013).

1.6 Scent-detection dogs in conservation

The use of scent-detection dogs for conservation first occurred in New Zealand in the 1890s, when they were used to detect kiwi (*Apteryx* spp.) and kākāpō (*Strigops habroptilus*) in efforts to conserve these species from predation and habitat loss from introduced mammals (Beebe *et al*., 2016; Browne *et al*., 2015). Until the early 1990s, conservation scent-detection dogs were used predominantly to detect endangered bird species, but their use has since been expanded into an array of uses within the field (Beebe *et al*., 2016). Scent-detection dogs have been used as a relatively safe, non-invasive method to successfully locate and monitor wildlife from larger animals such as bears (*Ursus* spp.) (Wasser *et al*., 2004), and tigers (*Panthera tigris*) (Kerley & Salkina, 2007) to reptiles such as the desert tortoise (*Gopherus agassizii*) (Cablk & Heaton, 2006), and smaller more cryptic species such as squirrels (*Sciurus* spp.) (Duggan *et al*., 2011) and brown hares (*Lepus europaeus*) (Karp, 2020). Dogs have also been trained to find both live organisms and carcasses of different species, as well as the scat of many more, becoming essential tools in wildlife surveys helping to establish population spread and density of these species (Beebe *et al*., 2016; Browne *et al*., 2006; Homan *et al.,* 2001).

They have also been used in the management of invasive species and diseases, having been trained to detect pest plants (e.g., spotted knapweed (*Centaurea stoebe*) (Goodwin *et al*., 2010) and animals (e.g., longhorn beetles (*Anoplophora glabripennis* and *A. chinensis*) (Arnesen & Rosell, 2021), as well as pathogens (Verma *et al*., 2021), and parasites (Alasaad *et al*., 2012) in plant and animal species in efforts to protect ecosystem biodiversity (Angle *et al*., 2016).

1.7 Applications and potential in New Zealand conservation

Scent-detection dogs have been widely used in New Zealand conservation by DOC for over 40 years (Browne *et al*., 2015; Department of Conservation, 2021). Dogs are commonly trained to locate endangered species such as kiwi (*Apteryx* spp.) and blue duck (*Hymenolaimus malacorhynchos*) (Browne *et al*., 2006; Robertson & Fraser, 2009), and have been successfully applied to find tuatara (*Sphenodon punctatus*) (Browne *et al*., 2015) and little penguins (*Eudyptula minor*) (Cargill *et al*., 2020). Dogs are also used to detect pest species such as Norway rats (*Rattus norvegicus)*, mice (*Mus musculus*) (Gsell *et al*., 2010) and Argentine ants (*Linepithema humile*) (Ward *et al*., 2014). As a result, DOC has established a Conservation Dog Programme and a certification process in which dogs are trained to detect either protected or pest species, and currently employ dog handler teams that are actively deployed throughout the country (Browne *et al*., 2015; Department of Conservation, 2021*a*).

Use of scent-detection dogs in conservation greatly increases the area that can be surveyed for wildlife, significantly reducing searching time and often with much higher accuracy than visual searching alone (Reed *et al*., 2011; Smith *et al*., 2001). Although most uses of scent-detection dogs are terrestrially based, there is potential for their use to be extended into aquatic based targets, for example, to whitebait species which lay their eggs within the dense riparian vegetation along waterways. It has been previously demonstrated that scent-detection dogs can locate invasive mussels attached to watercraft (Sawchuk, 2018), submerged cadavers (Osterkamp, 2011) and floating whale scat (Wasser *et al*., 2017), so it is plausible that scent dogs can be applied to New Zealand's endangered native, or even invasive, fish species.

1.8 Methodological considerations with scent detection

Despite the potential of scent-detection dogs, there are several methodological issues and limitations that need to be considered to ensure that the dogs are trained adequately and that results are valid. Factors such as environmental challenges, scent contamination,

methodological structure and handler cueing must be assessed and managed in order to ensure that dogs are trained well and provide meaningful results.

When working with scent tasks, there are several environmental factors that can affect scent detection performance. Some of these can be mitigated in a laboratory environment, however, in a field setting these factors are harder to manage. Temperature and humidity can change the scent profile of target odours, due to variation in the volatilization rate of VOCs (Angle *et al*., 2016; Naddeo *et al*., 2012). While this can be managed within laboratory environments, it is important that dogs are trained under varying conditions to ensure generalization of the target odour if the goal is for detection in the field (Moser *et al*., 2019; Oldenburg *et al*., 2016). Previous studies have noted that scent-detection dog effectiveness for finding target odours is impacted when there are variations in temperature and humidity or there are strong winds and rain (Reed *et al.*, 2011; Sargisson *et al*., 2012; Savidge *et al*., 2011). Under these conditions VOC release may be reduced, air currents can disperse the target odour and rain can dilute the scent profile (Reed *et al*., 2011; Wasser *et al*., 2004). These environmental challenges may be compensated for through handler training and adjusting searches so that the dog is assisted in finding the target based on the conditions, e.g., working downwind to help ensure success (Wasser *et al*., 2004).

When performing controlled scent detection experiments it is common to reuse samples multiple times during training, especially if the target scent is hard to acquire (Grimm-Seyfarth *et al*., 2019). As a result, it is easy for target scent contamination to occur, possibly changing the scent profile of the target odour and/or providing additional cues to the dogs (Cooper *et al*., 2014). This could lead to altered detection rates, dogs learning the wrong target odour and affecting the transferability of the target scent training from a controlled setting to a field setting (Cooper *et al*., 2014). When in controlled conditions, and especially in the initial stages of scent training, it is important that any contamination of the target scent is minimised to ensure proper learning. Dogs' olfactory sensitivity may be cued by something as simple as the scent carried on experimenter's gloves (Cooper *et al*., 2014), making it essential that diligence in sample preparation, proper cleaning, and glove changes are occurring during the initial stages of scent training, so that correct learning of the target scent is achieved. However, it has been noted in previous studies that if the end result of training is to be in a working field environment, that continued training should occur within a natural environment so that the dog can learn to detect the

target scent in the presence of background “noise” (Cablk & Heaton, 2006; Cooper *et al*., 2014).

Scent line-up arrangements are commonly used in experimental scent detection investigations and consist of presenting the dogs with samples of the target and non-target scents in a row. (Marchal *et al.*, 2016; Schoon, 2001). Dogs work along the line assessing each sample independently or accompanied by a handler and are expected to perform an indication response once the target scent is found (e.g., sit or lie down next to the sample) (Marchal *et al.*, 2016; Schoon, 2001). Although scent line-ups are considered effective, they are also labour intensive with data collected manually, leaving room for human error in data collection as well as during trials if reinforcement is unintentionally delivered for non-target behaviour (Ferry *et al*., 2019). Handler presence can lead to increased false indications or increased incorrect rejections of the target scent if the handler misses or incorrectly reinforces the dog, misinterprets dog behaviour or has not been adequately trained to avoid unintentional cueing (DeMatteo *et al*., 2019). When implementing scent line-up arrangements, it is important to avoid line-ups with a forced choice arrangement in which each line-up always contains a single target, as this is not representative of real-world scent-detection scenarios and can skew performance data (Edwards *et al*., 2017). In order to avoid this, it is important to implement line-up arrangements that have varied target amounts, (i.e., target is absent or the line-up contains more than one target) to establish an accurate representation of how the dog might perform in settings where quantities of the target are unknown (e.g., a field environment), and to ensure target scent learning has been established (Edwards *et al*., 2017).

Dogs are particularly receptive to human communication, able to interpret body language, respond to gestures and understand commands (Lazarowski *et al*., 2019). Because of this, dogs may be able to interpret any inadvertent cues given by the handler through body language, prompting the dog to give a response that the handler may expect. For example, Lit *et al*. (2011) demonstrated that handler beliefs towards the target location in scent detection tasks can influence the performance of their scent-detection dogs. Handlers were given false information regarding scent location, and it was reported that dogs falsely alerted more at these locations, compared to locations where the target scent was present. This highlights the importance of adequate handler training, blinded trials, and the need for scent-dogs to be able to work independently from their handlers (DeMatteo *et al*., 2019; Marchal *et al*., 2016; Wasser *et al*., 2004). This can be achieved through

carefully planned training in a controlled setting, ensuring greater success when the dog is transferred into a field setting (Marchal *et al*., 2016).

It is essential that handlers learn to be observant of dog behaviour whilst in controlled settings, to help ensure successful scent detection when the dog is working in field settings (DeMatteo *et al*., 2016). If handlers are not adequately observing dog behaviour, or have preconceived beliefs of target locations, it can result in reduced detection efficiency by dogs and missed targets (Cablk and Heaton, 2006; DeMatteo *et al*., 2016). Deficiencies in training can result in unreliability in dogs. For example, Engeman *et al*. (1998) found that training issues resulted in variable performance of alert behaviour by dogs trained to detect brown tree snakes (*Boiga irregularis*), thus affecting perceived effectiveness for using dogs for this purpose.

Due to the nature of conservation scent-detection work, dogs are often required to work in the field. Scent line-up arrangements are an effective way of training and transitioning dogs from controlled indoor training environments to the desired field setting and have been used in several studies (e.g., Smith *et al*., 2003; Wasser *et al*., 2004). Awareness surrounding possible drawbacks/limitations of handler presence and scent line-up designs must be considered, but these can be mitigated using appropriate training methods and experimental designs.

1.9 Project aim and structure

Due to the cultural and economic significance of whitebait species, and their continued population decline (Yungnickel *et al.*, 2020), more needs to be done to preserve and protect whitebait populations. Identification of spawning habitat is a key aspect to conserving whitebait species by maintenance of populations (Orchard & Schiel, 2021). However, visual surveys for spawning sites are time consuming and the locations of spawning areas can be difficult to predict as most species do not return to the same spawning site each year (Orchard & Schiel, 2021). Whitebait eggs are morphologically similar to eggs of other species (e.g., snails), and can be difficult for humans to visually differentiate. Scent-detection dogs may represent an efficient and effective way of locating whitebait nests. Dogs have demonstrated usefulness in other conservation contexts, having been trained to detect several different targets such as kiwi (Robertson & Fraser, 2009), bears (Wasser *et al*., 2004) and invasive mussels (Sawchuk, 2018), proving efficiency at locating both terrestrial and aquatic-based targets. If scent-detection dogs could demonstrate proficiency at detecting the odour of whitebait eggs, this could

lead to conservation applications that provide increased protection of spawning areas against disturbance and assist to fill life history knowledge gaps for culturally appreciated Galaxiid species.

The aim of this project was to investigate the ability of domestic dogs to detect and discriminate giant kōkopu eggs, with the goal that scent-detection dogs become a new tool for whitebait species conservation in New Zealand. Two approaches were taken to examine this research question. The first involved laboratory-based detection and discrimination of giant kōkopu eggs, consisting of six phases of training that began with simple discrimination, progressing to increasingly complicated line-ups and blind trial assessments of scent discrimination learning. The second approach was outdoor based detection and discrimination of giant kōkopu eggs, with line-ups following the same principles as previous training phases before progressing to trials where sample location was difficult to identify from a distance. It was hypothesised that, considering the success of dogs in other scent detection applications, that dogs would be able to discriminate whitebait eggs from other scents and this discrimination would also be able to be demonstrated in an outdoor setting.

Chapter 2

Methodology

2.1 Subjects

Pet dogs were recruited from throughout the Waikato region, New Zealand, using flyers, social media platforms and word of mouth. Criteria related to the dogs' food motivation, absence of separation anxiety and aggression, and comfort in a crate were used to screen and assess suitability for the project (see Appendix C). If these criteria were met, owners were provided details regarding the project and an opportunity to ask questions before giving written consent (Appendix B). Dogs were brought in for a probationary session and observed without the owner's presence, where initial project related training occurred.

The dogs used in this study had already undergone initial selection procedures and participated in previous studies conducted by the Scent Detection Research Group. Three of the five dogs had participated in a previous whitebait detection study and the other two had been involved in different studies that used an automated scent detection apparatus but did not meet the inclusion criteria for those projects. Information regarding the dogs that participated in this study can be found in Table 2.1.

Table 2.1 Subject information; NF represents neutered female, M represents entire male, F represents entire female

Subject	Breed	Age (years)	Sex
Rosie*	Border collie X bearded collie	5	NF
Bree*	Miniature poodle	4	NF
Puku*	German short-haired pointer X heading dog	3	M
Lexi	Springer spaniel X cocker spaniel	2	NF
Ella	Border collie	2	F

*- Participated in previous whitebait egg detection study

2.2 Animal ethics statement

The experiments in this study used domestic dogs and whitebait eggs (sp. giant kōkopu) and were approved by the University of Waikato Animal Ethics Committee (Protocol 1055). Dogs visited the University of Waikato Scent Detection Research Group facilities in accordance with a standard operating procedure (SOP) approved by the Animal Ethics Committee.

2.3 Study location and equipment

The study took place at the Scent Detection Research Group facility at the University of Waikato, Hamilton, New Zealand. The sample preparation room contained benches for workspace, a fridge, and a freezer for sample storage. The office had four large crates (1.8 m (height) x 1.2 m (width)) in which dogs were housed when they were not in the experimental room. Each crate contained a mat, blanket, and water bowl. Dogs had access to water for the duration of their time at the facility (approximately six hours or less each visit) and were taken outside by the researcher every 2 hours.

Figure 2.1 Office and dog housing area

For both indoor and outdoor trials dogs were given Possyum Supreme Dog Roll cut into approximately 2 cm x 2 cm squares as reinforcement, which was fed to the dogs by hand by the researcher. When not in the experimental room, dogs were given small amounts of Pedigree beef flavoured kibble as rewards for general good behaviour (e.g., going into crates, behaving well on walks). To prevent scent contamination, disposable gloves were

worn at all times whilst handling samples and were changed between handling different sample types, as well as between trials and for cleaning and handling of chemicals.

2.3.1 Indoor trials

The experimental room used in this study was 3.65 m x 6.65 m and had an air conditioner and two cameras set up. PVC tape was used on the floor to mark out sample placement in the scent line-up with six X's that were 75 cm apart (Figure 2.2).

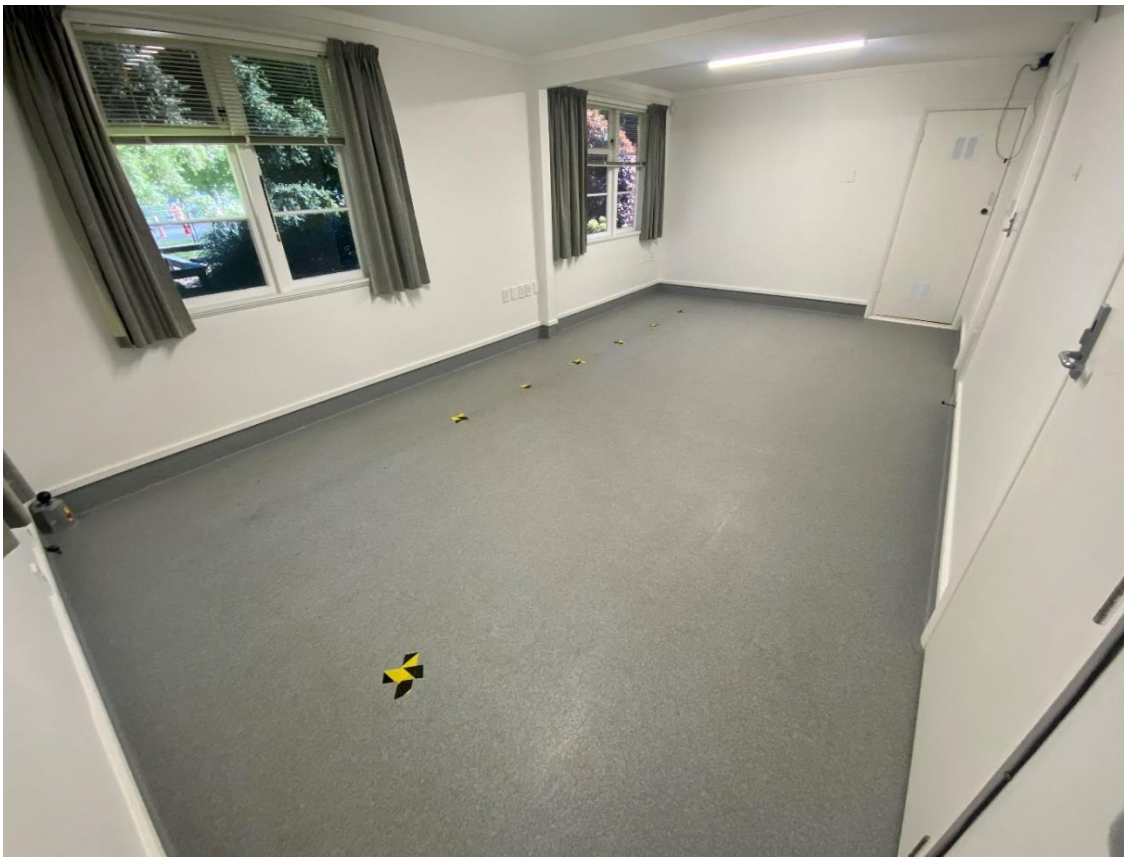

Figure 2.2 Indoor experimental room layout

Video footage was recorded using iSpy v7.2.1.0 on a computer set up in the office adjacent to the experimental room. Data were recorded on a wooden clipboard that was hung in the experimental room for ease of access by the researcher. Samples were presented in glass jars, on round stainless-steel cake pans (3.5 cm (height) x 21.5 cm (diameter)) and covered with either stainless steel colanders (16.5 cm (diameter) x 7 cm (height)) or stainless-steel bowls (14.5 cm (diameter) x 8 cm (height)) drilled with nine holes, that were placed upside down over the top of the samples (Figure 2.5 (b)).

2.3.2 Outdoor trials

Outdoor trials were recorded using a Sony HDR-PJ540E Full HD Handycam Camcorder on a tripod. Samples, treats, data sheets, clipboard, camera, and tripod were transported to the outdoor trial location. Temperature and humidity were recorded and measured using a hygrometer. Dogs were kept on a 7-m lunge lead for outdoor trials and were attached to hitching rings on a nearby fence between trials.

Samples were initially presented in the same way as the indoor trials (Figure 2.5 (b)). Later, samples were presented in small glass makeup containers (3.5 cm (width) x 2.5 cm (height)) with aluminium lids drilled with 5x 0.8mm holes (Figure 2.3).

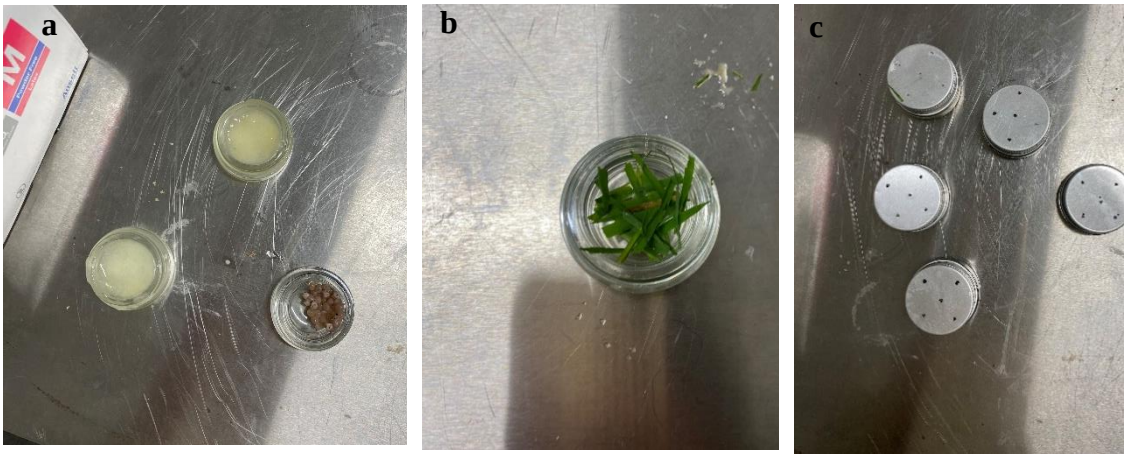

Figure 2.3 Whitebait, snail (a), and grass samples (b) inside the small glass containers used for the outdoor trials. (c) Samples covered with perforated lid for presentation in trials.

2.4 Sample collection

Fertilised giant kōkopu eggs were sourced from Manāki Premium New Zealand Whitebait, a commercial whitebait farming operation based in Warkworth, New Zealand. Eggs were distributed in 5 g lots into glass containers (3 cm (width) x 4 cm (height)) for storage and trial purposes (Figure 2.4). Each sample was assigned a sample number, which was displayed on the lid (Figure 2.4). New 5 g sets of whitebait samples were distributed halfway through the study. For the outdoor trials, 5 g lots of whitebait eggs were placed into six of the small glass makeup containers (Figure 2.3).

Non-target scents of snail eggs and grass were chosen, as snail eggs are visually similar to giant kōkopu eggs and may be mistaken for giant kōkopu eggs in the field, and grass

was chosen as it would be commonly encountered in the field. Garden snails (*Cantareus aspersus*) were collected from residential gardens and kept in terrariums within a temperature-controlled environment at the University of Waikato (animal ethics approval was not required to keep this species). The terrariums were filled with a base of soil to allow adequate nesting environment and were sprayed daily with water. Snails were provided with fresh lettuce as a food source, which was refreshed frequently. Daily checks of the soil for eggs were undertaken. When eggs were found, they were removed using a small metal spatula, rinsed under running water to remove any dirt, and placed into plastic containers (5 cm (width) x 6 cm (height)) for experimental use (Figure 2.4). Each container held one clutch of eggs (approx. 30 eggs) and was assigned a sample number that was displayed on the lid of the container. Care was taken to minimise any scent contamination occurring between the egg collector and the eggs, by ensuring that no direct contact was occurring. Once placed in the plastic containers, eggs were stored frozen until used in trials. For trials, eggs were thawed and presented in their plastic containers on the cake pans underneath the colanders/bowls. One clutch of eggs per glass makeup jar were transferred for use in the small jar outdoor trials. Grass samples were collected from the field next to the laboratory facility and each sample was approximately 15 g.

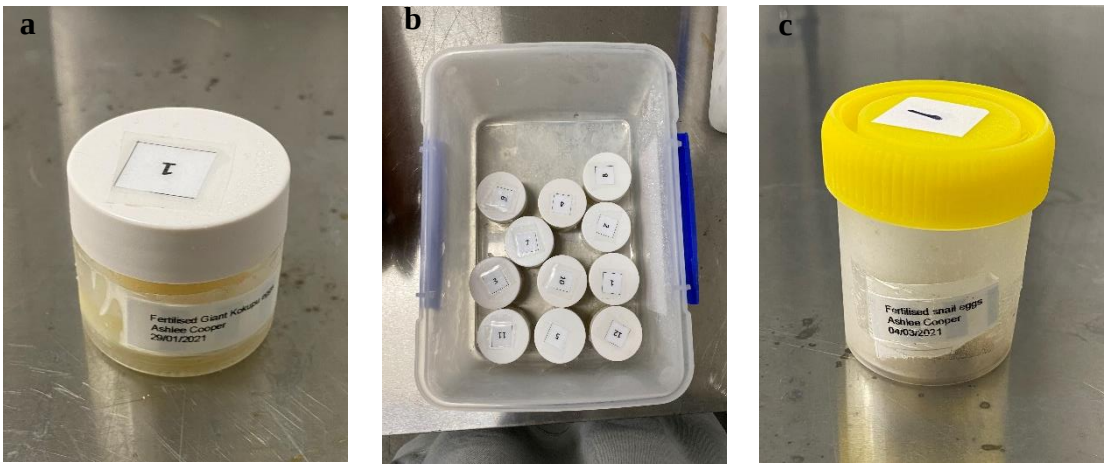

Figure 2.4 (a) Glass whitebait egg jar. (b) Storage container for whitebait jars, jars labelled for sample usage records. (c) Snail egg sample jar.

2.5 Sample preparation

Samples were prepared in the morning of the day of testing. Snail eggs and the giant kōkopu eggs were kept in separate enclosed plastic containers whilst not in use. On the

day of testing, one container of snail eggs was removed from the freezer by the researcher and placed onto one of the pre-cleaned metal cake pans and covered with an upside down colander (Figure 2.5). The snail eggs were taken to the experiment room and set on the ground to thaw before being used in the line-up. The same procedure was followed with the whitebait eggs to ensure that they were adequately defrosted by the time trials begun. The assigned sample number for both snail and whitebait eggs was recorded to keep record of how many times samples were being re-used. Whitebait egg and snail egg samples were selected at random. The same snail egg samples were rotated through the duration of the study due to difficulty in harvesting new snail eggs. Grass samples were gathered from outside, placed into the pan/colander set-up and put into the experimental room. Care was taken to ensure grass was not visible on the cake pan. Blank samples were the pan/colander set-up, with nothing underneath.

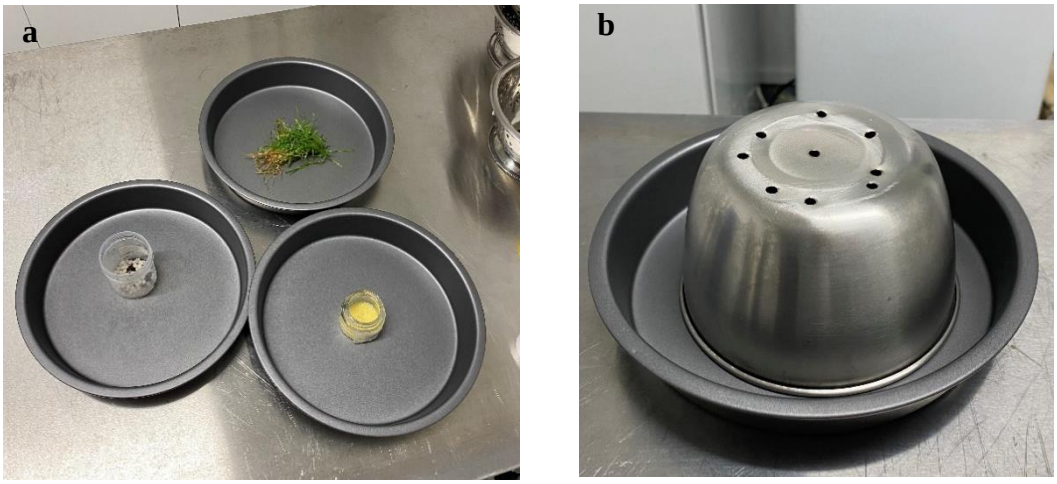

Figure 2.5 (a) Samples presented on cake pan. (b) Sample set-up for presentation in trials.

2.6 Cleaning

Once trials were finished, equipment and samples were collected and cleaned. The outside of the snail egg and whitebait sample containers were wiped with a 60% isopropanol (IPA) solution, before being placed into assigned freezer containers and frozen. Cake tins, colanders, glass jars (containing blank or grass samples) and metal lids were washed in a sink of hot water and a dissolved Sunlight Power Max dishwashing tablets and left to dry on a metal bench that had been wiped down with 60% IPA. Once the washed equipment was dry, it was wiped with IPA and stored.

The experiment room was swept after each session and vacuumed and steam-mopped once a week. Dog blankets were washed once a week, and water bowls after each session. Crates and mats were sprayed with Virkon solution and wiped with a paper towel after each day.

2.7 Experimental procedure and training

Experimental days were made up of three sessions per day per dog. A session usually consisted of 6-10 trials, with a trial being a single run down the line-up. Refer to Appendix D for example videos for Phases 3, 5 and 6. Dogs would attend up to 3 days per week. Days were usually split into either morning or afternoon sessions, with most dogs only spending half days at the laboratory. Morning sessions typically ran from 8 am to 12 pm and afternoon sessions from 1 pm to 4:30 pm, depending on flexibility of owners to collect their dogs. Sessions were kept to between 10-15 minutes, as anything longer resulted in noticeable loss of interest from some of the dogs. Training was split into different phases that increased in complexity as the conditions increasingly approximated field conditions. An SOP of the experimental procedure can be found in Appendix D

2.7.1 Phase 1: Shaping sniff and indication response

Individual dogs were habituated to the researcher and the testing environment before training was begun. The researcher worked to form a positive relationship with the dog through verbal and food reinforcement and physical touch. Once the dog displayed signs of habituation (e.g., relaxed body language, wagging tail) to the researcher and the testing environment, it was determined that they would be able to begin training sessions.

Dogs were presented with a positive sample from the start of training, where sniff and indication response were established. Other studies have started sniff and indication training by only presenting a high value reward (e.g., a hotdog) in a line-up, to encourage active sniffing and establish a search pattern, before a target sample is introduced (e.g., Marchal et al., 2016; Kerley & Salkina, 2007). However, for the purpose of this study, it was decided that exposure to the target scent from the beginning would provide effective, ongoing scent association by the dog and help to prevent confusion as to what scent would be reinforced. Successive approximations were used to shape sample sniffing response and to begin training of the "go find" command, before training the indication response. Three of the dogs had previous training experience with giant kōkopu scent and were familiar with the use of a "go find" command.

A single positive sample was placed in the experimental room to begin scent association training. The dog and researcher would enter the room and the researcher, with their back against a wall facing the sample, would tell the dog to "go find". Initially, the dog was reinforced by hand-delivery of food and verbal praise from the researcher for approaching, interacting or sniffing the sample. This later transitioned to only receiving reinforcement for behaviours that were closer to the target sniffing response. Eventually, dogs were only reinforced when their nose was within 10 cm of the sample. If the dog did not approach the sample after 1 minute, the researcher would prompt the dog by pointing and/or approaching the sample whilst giving the "go find" command. When the dog demonstrated investigation of the sample by putting their nose within 2 cm of the sample, a treat would be given, and the prompt phased out.

A reliable sniff of the sample was defined as the nose being within 2 cm of the colander or tin for five out of six trials. The session began when the "go find" command was given and ended once 10 minutes had elapsed. Once the dog was demonstrating a reliable sniffing response, the positive indication response of a sit-stay was shaped. This was achieved by giving the "go find" command and once the dog was sniffing the sample, the researcher would approach the dog and tell them to "sit." Once the dog was sitting, the researcher would give reinforcement and the trial was reset. This was repeated approximately three times before the "sit" command was phased out. Trials were repeated until the dog was sitting reliably after sniffing the sample without the use of the "sit" command (five out of six trials).

To prepare dogs to work in a field setting, an extended stay and delayed reinforcement needed to be integrated into training. Once reliable sniffing and sitting was achieved, a stay response was shaped. For these trials, the dog was told to "go find". Once sitting at the sample, the researcher would approach and tell the dog to "stay", then deliver reinforcement. The distance between dog and researcher was increased until the researcher remained at the observation position, while the dog stayed at the sample. Once the dog was reliably (three consecutive trials) staying at the sample, the "stay" command was phased out and the indication response achieved.

The duration of the stay was then extended. The dog was given the "go find" command, and once the dog indicated at the sample, the researcher delayed before moving to reinforce the dog at the sample. Delay durations started at 1 s, proceeding to intervals of 5 s, 10 s, 15 s and 20 s before the researcher moved to reinforce. The dog had to remain

in a sit-stay for three consecutive trials at each interval before moving to the next one, progressing to the next phase once this was achieved at 20 s delay.

Throughout this phase, the researcher would touch, move, and look underneath the colander before delivering reinforcement. This was in preparation for field conditions, where a handler may need to check that eggs are present before reinforcing a dog's indication behaviour.

2.7.2 Phase 2: Introduction of blank samples

To begin teaching discrimination and to ensure proper sniffing behaviour was occurring, blank samples were introduced into trials. These trials were 'forced choice' trials, where the dog was presented with two or more options and had to choose the right one (i.e., the whitebait eggs). There were 10 trials per session and three sessions per day for this phase. The trials began with one positive sample and one blank sample placed in line with each other, approximately 75 cm apart. Sample position was established numerically running left to right from the point of entry in the room, and order of samples was determined using the RAND function of Microsoft Excel. The dog was brought in and told to "sit" and "stay" at a starting position that faced them directly towards the presented samples. The researcher would position themselves at an observation point and tell the dog to "go find". In these trials and all future trials, the researcher maintained neutral body language and avoided eye contact with the dog in efforts to prevent unconscious cueing. Once the dog indicated a choice, the researcher would delay up to 5 s before moving to the dog to encourage clear indication. If the indication was correct, the dog would receive praise and treats; if the indication was wrong, the dog was reset to the starting position and moved to the next trial, with the researcher remaining neutral in body language and voice. The dogs were trained to remain in the sit-stay starting position whilst the researcher reordered samples, and then given the release "go find" command. If the dog did not make a choice after 20 s, or would not make a reliable choice (i.e., moving to and sitting at different samples), the trial was ended and the dog would be reset back to the starting position. Once the session was finished, there was a 5–10-minute interval before the next session started. Correct and incorrect indications were recorded, and a criterion of 80% correct indications (8 correct in the 10 trials) for two out of the three sessions was required before an additional blank sample was added. The same procedure was followed with two blank samples and one positive sample, with the dogs having to meet the 80% correct criteria for two out of three sessions, before moving to the next phase.

2.7.3 Phase 3: Discrimination and distractors

Non-target scents were introduced into the sessions to further refine scent discrimination. Trials were moved away from forced-choice trials in which there were always one target sample only presented with blanks, to line-up-based trials where the target was presented along with distractor scents (Figure 2.6). To begin, trials consisted of one positive sample, two blank samples and a snail egg sample. The samples were placed in marked positions 75 cm apart in a line down the room. Sample position within the line-up was labelled numerically, with sample order determined using the RAND function of Microsoft Excel. Dogs were positioned at a starting point looking down the line. The researcher would observe the trial from a position next to the starting position of the dog, to prevent unintentional cueing and to simulate what would occur in a field setting. The dog was told to "go find" and reinforced for indicating on the positive sample or reset if an indication was made on a non-target sample, or if there was no indication. Dogs had to achieve an 80% correct positive indication rate across all samples for two out of three sessions, before the grass sample was added to the line-up. Once the 80% correct positive indication rate was achieved for line-ups containing the positive sample, two blanks, snail eggs and grass the dog progressed to the next phase.

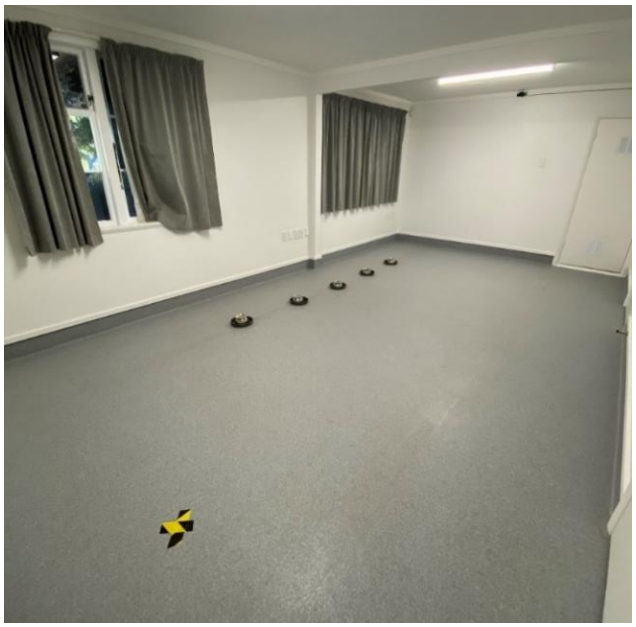

Figure 2.6 Samples arranged in a line-up

2.7.4 Phase 4: Training search pattern

Dogs were trained to systematically work down the line-up to ensure all scents were investigated, and to establish a search pattern that would be used in the field. In this phase researcher accompaniment of the dog along the line-up was integrated, as preparation for field conditions. Line-ups consisted of the same five sample types (one positive, two blanks, one snail egg, one grass) used in the previous phase. To begin, positive samples were placed at the end position furthest from the dog to encourage searching of the entire line-up. This arrangement was based on methods used by Rosell *et al*. (2020) in their study with beaver scent. For this initial search pattern training, snail egg and grass samples remained in the first two positions of the line-up, and no re-arrangements of the samples between trials occurred. The researcher and dog began at the starting position, and the dog was given the "go find" command. Walking alongside the dog, the researcher would prompt systematic investigation of the samples using pointing, gestures, or verbal prompts. The dog was reinforced for correct indication of the positive sample and moved to the next trial. If an incorrect indication was made, no reinforcement was given, and the dog was encouraged to continue investigating down the line. If no additional indications were made, the trial was ended, trial reset, and the dog brought back to the starting position. Investigation prompts by the researcher were phased in and out as required and systematic search performance was evaluated after each session (10 trials). If the dog was independently systematically searching each sample in the line-up for 8 out of 10 trials, the position of the positive, blank, grass, and snail egg samples in the line-up was randomised. If the dog was not independently systematically searching each position in the line-up the dog would repeat another session with the positive in the end position and the handler prompting as needed until the criteria was met.

In the randomised sample position trials, the same basic procedure was followed, but the dog was required to systematically search each sample and perform a correct indication response. If the dog was not systematically searching each sample, the trial was considered incorrect, and the dog moved to the next trial. From the starting position, the dog would be given the release command, with the researcher following slightly behind. Once the dog performed the indication response, the researcher would stop and stand for 5 s before either giving reinforcement for a correct indication or prompting the dog to continue investigating down the line. Dogs were expected to stop and indicate once they reached the positive sample in the line-up and could only investigate samples adjacent to each other, to prevent movement back and forth down the line-up, as this would not be

ideal search behaviour in the field. It was found in early trials that the dogs expected the positive sample to be at the end of the line-up, working quickly down the line without proper investigation. As a result, verbal prompts were integrated to slow down the dogs and encourage proper sample investigation to prevent the positive sample from being skipped. If the dog had to be prompted to the location of the positive sample, the trial was marked as incorrect. In these randomised position systematic search trials, dogs had to be consistently systematically searching (8 out of 10 trials) and achieve an 80% correct indication rate for two out of three sessions to be able to progress to the next phase.

2.7.5 Phase 5: Introduction of additional positives and randomisation of positive sample amounts

Positive sample numbers were increased from one to two, to encourage continued working down the line-up and to teach the dog that there may be more than one target within the line-up. Positive sample number was later varied within the trials to between 0-2, to prepare dogs for field conditions in which the number of targets is not fixed and to train dogs to correctly reject all samples when no targets are present. In this phase dog responses to each sample position were recorded to determine hit rate, correct rejection and false alarm rates. Hit rates represent indication responses (sitting) made when target samples are presented, whereas false alarm rates represent indication responses made when non-target samples are presented. Comparing hit rates with false alarm rates provides information on accuracy with positive and negative samples.

In the initial trials, one positive sample was positioned at the end of the line-up (furthest from the dog), and a second positive sample was positioned randomly elsewhere in the line-up. The dog was positioned next to the researcher in the starting position and given the “go find” command, with the researcher following slightly behind. Once the first indication was made the researcher would stop and wait 5 s. If correct, the dog was reinforced and given the “go find” command again and prompted (gestured or pointing) down the line; if incorrect, the dog was given the command again and prompted down the line. Reinforcement was given if a correct indication was made when the second positive sample was encountered. Initially, if positive samples were missed, the dog would be prompted to reinvestigate samples to continue to encourage scent association learning. These sessions consisted of 10 trials, with three sessions completed a day. A daily average of ≥90% hit rate (proportion of correctly indicated positives) and ≤10% false alarm rate (proportion of incorrect indications) was required before the dog was moved to having

both positive samples randomised. The same procedure was repeated with both positive samples randomised, with sample order established using the RAND function on Excel. Once the criteria of a daily average ≥90% hit rate and ≤10% false alarm rate was met, the dog was progressed to trials where the number of positive samples were varied, including being absent from the line ups.

Due to the added complexity of varied positive trials, sessions were reduced to six trials, as there was a noticeable decline in dog interest due to the session duration. Positive sample numbers were varied to either two, one or none per trial. Each session was made up of three double positive trials, two single positive trials, and one zero positive trial, or five double positive trials, four single positive trials and one zero positive trial in the earlier days of this phase before trials per session was adjusted; with the order of the trials randomised within the session. Once the dog achieved a daily average ≥90% hit rate and ≤10% false alarm rate, the session structure was changed to consist of two double positive trials, two single positive trials and two zero positive trials (or four double positive trials, four single positive trials, two zero positive trials in the initial days). Once the dog met criteria (daily average ≥90% hit rate and ≤10% false alarm rate) in these sessions, it was progressed to the next phase.

2.7.6 Phase 6: Single blind trials

By this phase, dogs were reliably and consistently identifying and discriminating whitebait eggs in the line-up, but the researcher was aware of the target locations during training up to this point. Therefore, blind trials were integrated to ensure true discrimination and that unintentional cueing was not occurring. The structure of the blind trials was the same as Phase 5, except the order of the line-ups and distribution of each trial type was determined by an assistant. The researcher and the dog would wait in a separate room, with no view of the assistant or the experimental room. The assistant would determine sample order and arrange the samples within the experimental room. The assistant would then watch the trials on a computer set up in the office that was connected to two cameras within the experimental room, recording the dog response at each position in the line-up. The assistant would tell the researcher to enter, the dog and researcher would enter the room moving straight to the starting position. The dog would be told to "sit" at the starting point, before being given the release command and working down the line with the researcher following. When a dog indicated, the researcher would pause 5 s, check the sample beneath the colander and give reinforcement if the indication was correct, before prompting the dog to continue down the line. Once the trial ended,

the researcher and dog would leave the room and the assistant would re-enter and arrange the samples for the next trial. This process was repeated for each of the six trials in the three sessions. Once the dog received a daily average ≥90% hit rate and ≤10% false alarm rate, it was determined that scent discrimination was occurring, and the dog could progress to outdoor trials.

2.7.7 Phase 7: Transition to outdoor setting

With scent discrimination occurring, line-ups were moved to an outdoor setting to assess how dogs would perform in a less controlled, more field-like environment. A flat, grassed, relatively low trafficked area that ran along a building was picked for outdoor trials (Figure 2.7). A camera and tripod were set up, with the camera view encompassing the testing area, temperature and humidity were also recorded. The samples and other equipment were transported to the testing area using a cart, with positive samples kept separate from the other samples during transport. The dog was kept on a long lead for the entirety of the time outside and connected to a hitching ring while the researcher rearranged samples between trials. Trials followed the same principles as in Phase 5 (non-blind), with the samples presented in the same cake pan and colander set-up that had been used indoors. Samples were arranged at varying distances in a line alongside the building, which acted as a pseudo-riverbank. The dog was unhitched and led to the start of the line-up and given the command to "go find", running on a loose lead along the line. Reinforcement was given for correct indications, and responses to each sample in the line-up were recorded. The dog was reattached to the hitching ring and the researcher would rearrange samples for the next trial, repeating for the six trials for three sessions. Once the dog met the daily average ≥90% hit rate and ≤10% false alarm rate, it was moved to small jar outdoor trials.

Figure 2.7 Outdoor trial set-up

The small jar outdoor trials followed the same principles as the previous trials in this phase, except samples were presented in small glass makeup containers with perforated metal lids (Figure 2.8). Small containers were used to teach the dog to not simply approach the large, easily identifiable colanders, and instead search for the target odour. The same criteria of daily average ≥90 hit rate and ≤10% false alarm rate needed to be met for the stage to be considered finished.

Figure 2.8 Samples in small glass containers presented in the grass

2.8 Data collection and analysis

Microsoft Excel was used for data recording, analysis, statistics, and graph creation.

2.8.1 Phases 1-4

Data were recorded for number of prompts used and correct or incorrect whitebait egg identification was recorded. Data were summarised into accuracy, summarising correct responses in each session and establishing a percentage. Dogs had to meet accuracy criteria of >80% accuracy for two out of three sessions in order to determine if dogs were achieving required scent learning. Graphs were created from both the daily correct

response averages, as well as the percentage correct in the three sessions and used for analysis.

Accuracy (%) = Number of correct trials/10 x 100

2.8.2 Phases 5-7

Data were recorded for responses at each sample position for indications and correct or incorrect responses. For example, if the dog sat at a positive sample, it was recorded as a correct response and an indication. If the dog sat at a non-target sample, it was recorded as an incorrect response and an indication. From these data, averages for each session, as well as daily averages could be established for hit rates of the target samples, and correct rejection rates and false alarm rates of each non-target sample type and combined overall correct rejection and false alarm rates of non-target samples could be determined, graphed and analysed.

Hit rate (%) = number of correctly indicated positives or hits/total number of positive samples x 100

Correct rejection (%) = number of correctly rejected non-target samples/number of non-target samples x 100

False alarm (%) = 100 – correct rejection of non-target sample

2.8.3 Position bias

False alarm data at each sample position for phases 5 (non-blind) and 6 (blind) for each dog were gathered and proportions of responding compiled into a heat map for visual determination as to whether dogs had any position biases.

Chapter 3
Results

3.1 Preliminary training stages (Phases 1-2)

All five dogs that participated in the preliminary training stages met the mastery criteria (≥80% accuracy for two out of three sessions). Four dogs required between 1-3 sessions to learn the indication response, while one dog required six sessions. The dogs were able to successfully discriminate giant kōkopu eggs from an increasing number of blank samples. On average, it took 28.2 (*SE* ± 1.82) sessions for the dogs to progress from one to two blank samples, with the progression criteria of 80% correct for two out of three sessions. Once progressed to two blank samples, Lexi (Figure 3.1) took nine sessions before meeting criteria to move to Phase 4, with the other dogs requiring between 27-60 sessions before meeting criteria (Figures 3.3-3.5). All dogs had an accuracy of 40-60% at the beginning of training, which increased as training went on, except for Ella (Figure 3.2), who was slower to progress before her accuracy increased to the required 80% criteria. Performance generally decreased and became more variable when moving from one blank sample to two blank samples. Average variation of performance between sessions generally differed by <10%, except for Lexi whose accuracy was worse in the third session by >15% on average ($\bar{x}$ = 54%) than in the first ($\bar{x}$ = 65%) and second sessions ($\bar{x}$ = 70%).

3.2 Discrimination training (Phase 3)

Phase 3 assessed whether the dogs were able to successfully discriminate the target scent (whitebait eggs) from other distractor scents (snail eggs, grass). Dogs had to meet a success criterion of ≥80% accuracy for two out of three sessions to progress to additional samples. Four of the five dogs successfully progressed from the addition of snail eggs and grass samples, taking 6-15 sessions to meet the criterion required to move to the next phase. Lexi (Figure 3.1) and Puku (Figure 3.2) met criterion within the first day (three sessions) of each introduction of the snail and grass samples, progressing to the next phase in six sessions. Rosie (Figure 3.5) and Bree (Figure 3.4) required more sessions after the introduction of snail eggs, progressing to grass after 12 and 15 sessions, respectively. Once introduced to grass samples, both Rosie and Bree met the criterion in 1 day (three

sessions) and were able to progress to the next phase. However, Ella did not meet criteria to progress from the snail samples (Figure 3.2). After 33 sessions on the snail samples, Ella was regressed to Phase 2, where she met criteria for one blank and one positive trials; however, after consistently not meeting criteria for 2 days (achieving <50% accuracy), she was withdrawn from the study due to lack of motivation.

3.3 Search pattern training (Phase 4)

The purpose of this phase was to establish a consistent search pattern along the sample line-up, to ensure proper investigation of each sample was occurring. Criteria to progress from this phase was based on systematic search performance. Dogs had to consistently search each sample systematically and independently and indicate on the target sample for 8 out of 10 trials in the session. All four of the dogs met criteria to progress from this phase, and progression from this phase took between 9-45 sessions depending on the dog. In the first step of training, the positive sample remained in the end position of the line-up, and once criteria were met, the positive sample position was randomised. It took the dogs between 4-39 sessions to progress to randomised positive position. In the randomised position trials, the dogs had to search each sample position systematically and independently, as well as achieve an ≥80% accuracy for two out of three sessions. It took the dogs between 5-14 sessions to be eligible to move to the next phase.

3.4 Additional positives and randomisation (Phase 5)

The purpose of this phase was to encourage continued searching of the line-up and to teach the dogs that there may not always be a positive sample present in the line-up. This was to prevent false alarm indications, as well as to simulate field conditions where a positive find is not guaranteed. The number of positive samples was increased to two for the initial portion of this stage and was then randomised between 0-2 per trial.

Criteria to progress in this phase were no longer based on accuracy, but on combined hit and false alarm rates. Dogs had to meet mastery criteria of ≥90% daily average hit rate (HR) and ≤10% daily average false alarm rate (FA) for a phase change to occur. Comparing hit rates with false alarm rates provide information on accuracy with positive and negative samples and allows for more precise analysis of dog performance.

Training for the dogs was maintained at this stage whilst blind testing arrangements were being made, so progression from this phase was delayed. All four dogs met criteria multiple times during this phase. Lexi maintained criteria for four sessions in a row and

then again for six sessions (Figure 3.1). Puku met criteria multiple times over this phase, but once progression could occur, Puku was consistently performing below criteria, so he was excluded from further trials (Figure 3.3). Bree (Figure 3.4) and Rosie (Figure 3.5) were less consistent at maintaining criteria, but still performed above chance, fluctuating between 75-96% hit rate and 0-13% false alarm rate until progression from this phase was possible. Lexi, Bree, and Rosie spent between 13-16 days at this phase, and Puku spent 22 days in this phase.

3.5 Blind trials (Phase 6)

Blind trials were used to assess if dogs were demonstrating target scent discrimination and to determine if any unintentional cueing from the researcher was occurring. Lexi and Rosie met the ≥90% hit rate and ≤10% false alarm rate criteria, spending between 4-8 days in this phase. False alarm responding increased in this phase for all dogs. Rosie and Bree indicated incorrectly the most frequently on snail samples; Rosie's average false alarm responding to snail samples over all sessions was 35.2% (Figure 3.5) and Bree's was 27.1% (Figure 3.4). Lexi incorrectly indicated on blank samples the most, emitting an overall average false alarm rate of 14.4% on these samples (Figure 3.1). As the blind sessions progressed, there was a gradual increase in false alarm responding by Bree (Figure 3.4), at the highest point reaching 36.3%, which began decrease to 30.2% by the final day. Rosie, also, had a rise in false alarm performance, 39.8% at the highest, but this gradually decreased to 8.8% by the final day. Bree's hit rate performance was varied but generally increased over the days, at the lowest point being 37.5% and the highest being 83.3%. Rosie also had variation in hit rate during this phase (Figure 3.5), meeting average daily hit rate criteria (≥90%) during two days of this phase, but was unable to progress on the first day due to not achieving false alarm criteria (≤10%), with a FA rate of 32.25%. Lexi (Figure 3.1) was also unable to progress from this phase for the same reason, as her FA rate was 13.9% on the first day she met hit rate criteria (achieving 100% HR). Dogs were required to meet both hit rate and false alarm criteria before being able to progress, to ensure discrimination accuracy and to establish desirable responding in circumstances that more closely resemble field search conditions. The second day Lexi achieved hit rate criteria (91.7% HR), her false alarm rate had fallen to 1.8% and so she was able to progress to the outdoor trials. On the final day of the project Rosie met both hit rate (91.7% HR) and false alarm (8.8% FA) progression criteria but she was unable to progress outdoors due to the cessation of the project.

3.6 Outside trials (Phase 7)

Trials were transitioned outside in a progression to field-like conditions and to assess discrimination performance outside of a laboratory environment. Lexi was the only dog that progressed to the outside trials. Progression criteria of ≥90% hit rate and ≤10% false alarm rate was required to move to the small jar trials. Lexi required 2 days to meet criteria in the initial line-up trials in this phase (Figure 3.1). A ≤10% false alarm rate was maintained across these days, and hit rate progressed from 87.5% to 95.8%. More false alarm responses were made on average to blank samples ($\bar{x}$ = 8.6%), than snail egg ($\bar{x}$ = 2.8%) and grass ($\bar{x}$ =5.6%) samples, in this stage of the outdoor trials.

3.6.1 Outside small jar trials

Samples were transitioned to small glass jars and presented in the line-up, in place of the large colander/pan set-up that had been used throughout the previous phases of training. The aim of this transition was to assess dog discrimination and search performance when samples were harder to identify from a distance in order to encourage active searching from the dog. Lexi required 5 days on the small jar trials before meeting hit rate and false alarm criteria. The first day of these trials had an increase of overall false alarm responding to 14.5%, with false alarm responding to the grass samples rising to 22.2%. However, for each subsequent day, average false alarm responding was ≤2.3% until Lexi met the hit rate criteria. Hit rate was reduced to 75% before steadily increasing. The hit rate and false alarm progression criteria were met on the final day of the project, where Lexi achieved a 91.7% hit rate and a 1.85% false alarm rate but was unable to progress to any further training due to the cessation of the project (Figure 3.1).

3.7 Position bias analysis

To establish if any dogs were exhibiting sample position bias the proportion of false alarm responses for each sample position for Phases 5 (non-blind) and 6 (blind) were calculated (Figure 3.6). Assuming that the dogs had no bias for sample position, the expected proportion of false alarm responses would be approximately 0.17. However, large deviations from this value would suggest bias either for (higher values) or against (lower values) sample positions. In general, there was a notable bias towards the 6th position by Rosie and Bree with corresponding avoidance of the 1st position. Bree also had a bias towards the 5th sample position (0.34) in the Phase 5 non-blind trials. Puku and Lexi had exhibited relatively weak position bias in the Phase 5 trials.

In Phase 6, there appeared to be moderate position biases for all dogs at the 6th position of the line-up (Figure 3.6), with the proportion of false alarm responses at the 6th position ranging from 0.25-0.31. Rosie also had a higher proportion of false alarms at the 2nd position with a corresponding decrease in false alarms at the 5th position, suggesting there was bias towards the 2nd position and avoidance of the 5th position. Bree had a higher rate of false alarm responding occurring at the 6th (0.31) position, but the false alarm responses from positions 2-5 were close to the expected proportions. Lexi's false alarm responding was relatively evenly spread throughout each position, with false alarm responding on a position not exceeding 0.25 (Figure 3.6). Overall, all dogs appeared to have a bias towards the 6th position with a corresponding aversion to the 1st position, although this was less pronounced in the Phase 6 trials compared to the Phase 5 trials.

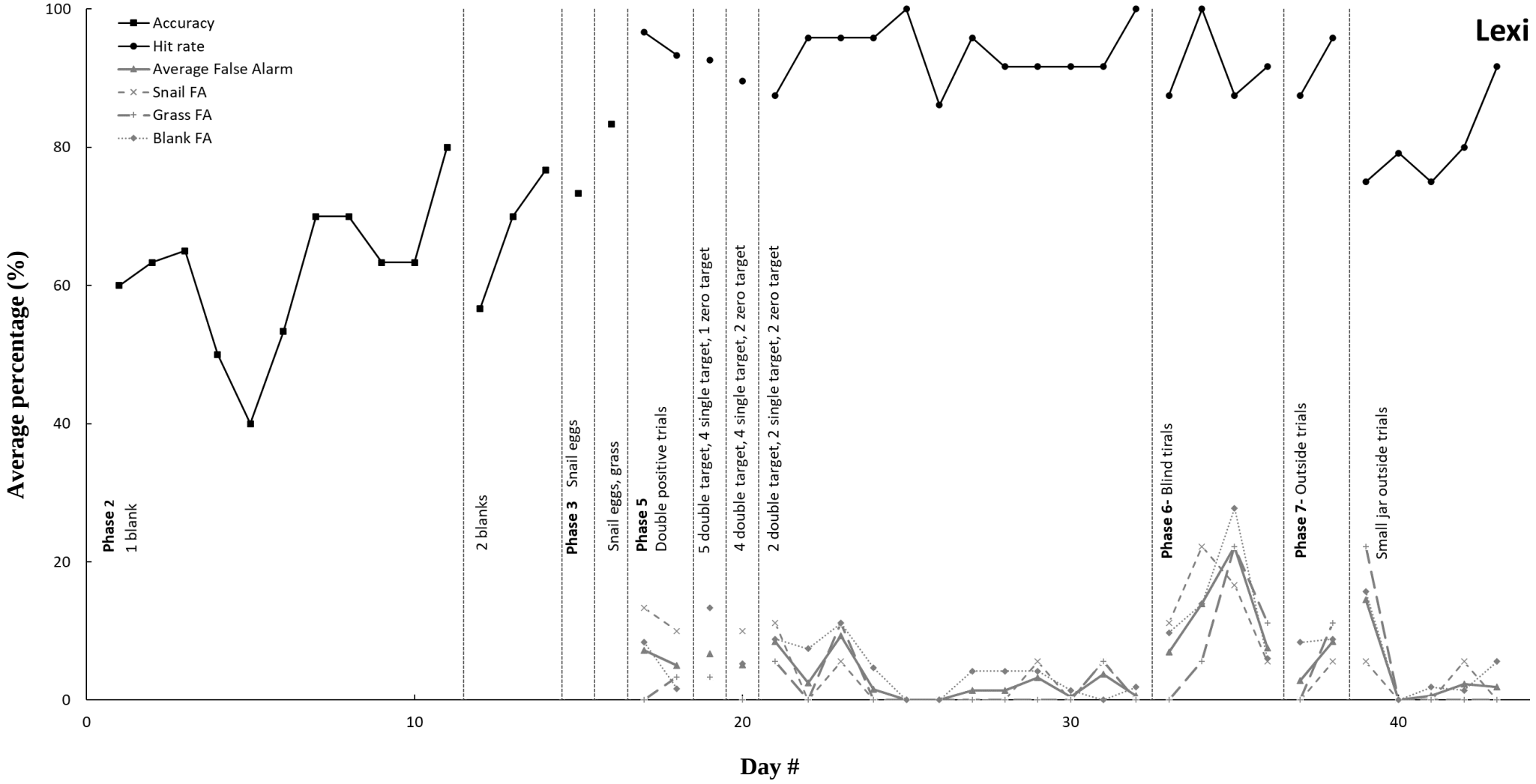

Figure 3.1 Lexi's daily average accuracy (percentage of trials correct) in Phases 2 and 3 and daily average hit rate and false alarm rate across Phases 5 to 7, including false alarm rate for each sample type

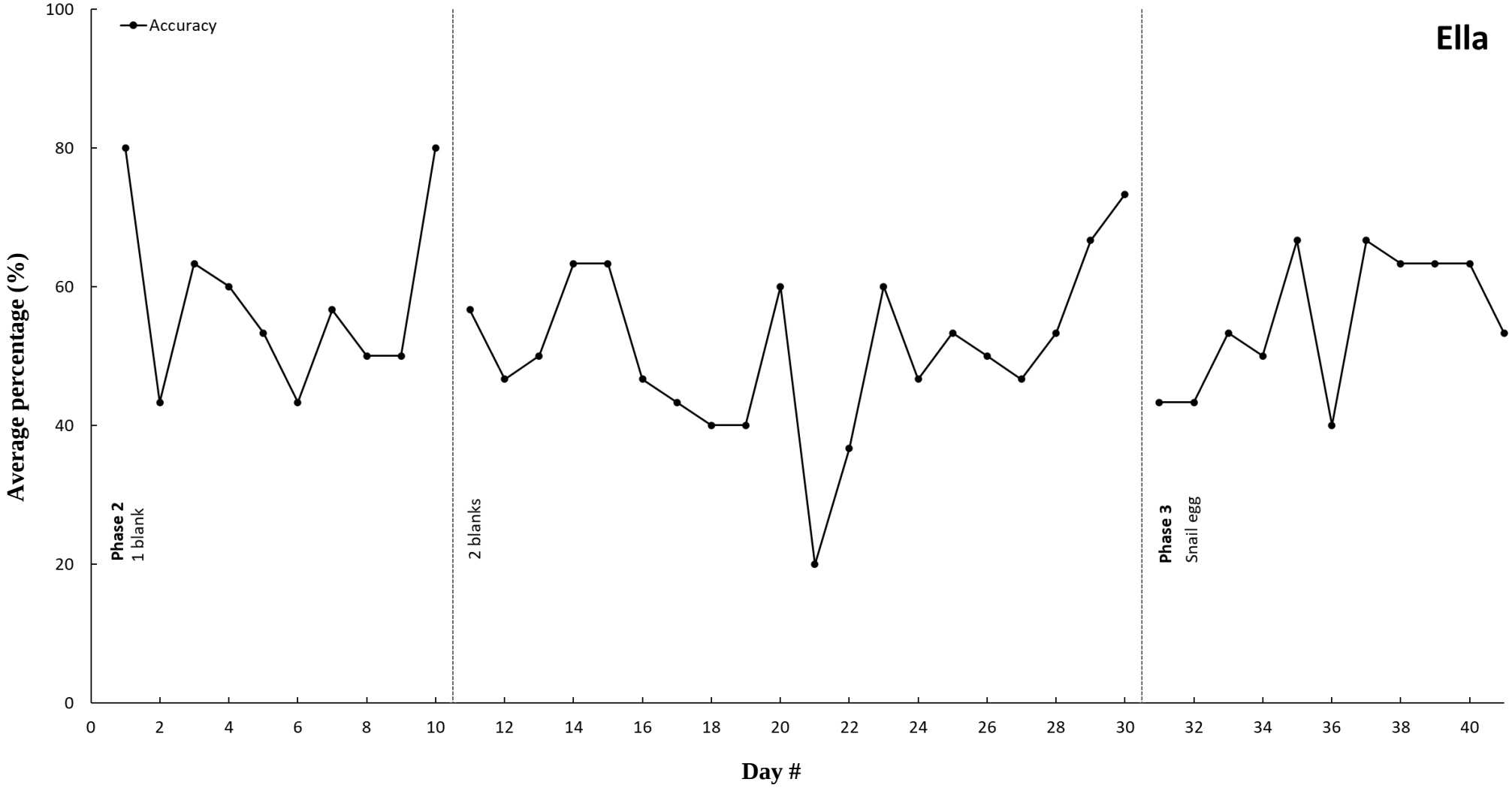

Figure 3.2 Ella's daily average accuracy (percentage of trials correct) in Phases 2 and 3

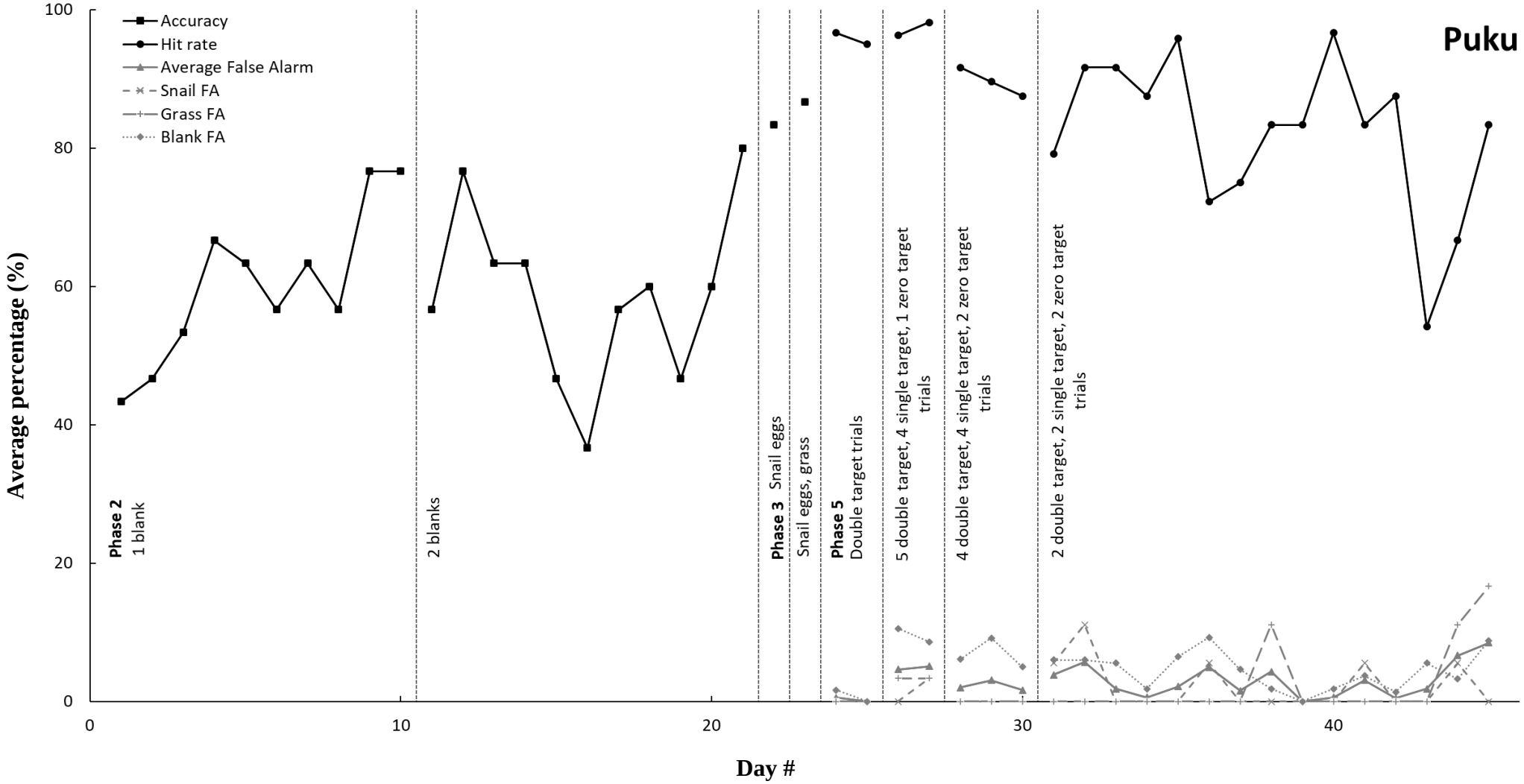

Figure 3.3 Puku's daily average accuracy (percentage of trials correct) in Phases 2 and 3 and daily average hit rate and false alarm rate across Phase 5, including false alarm rate for each sample type

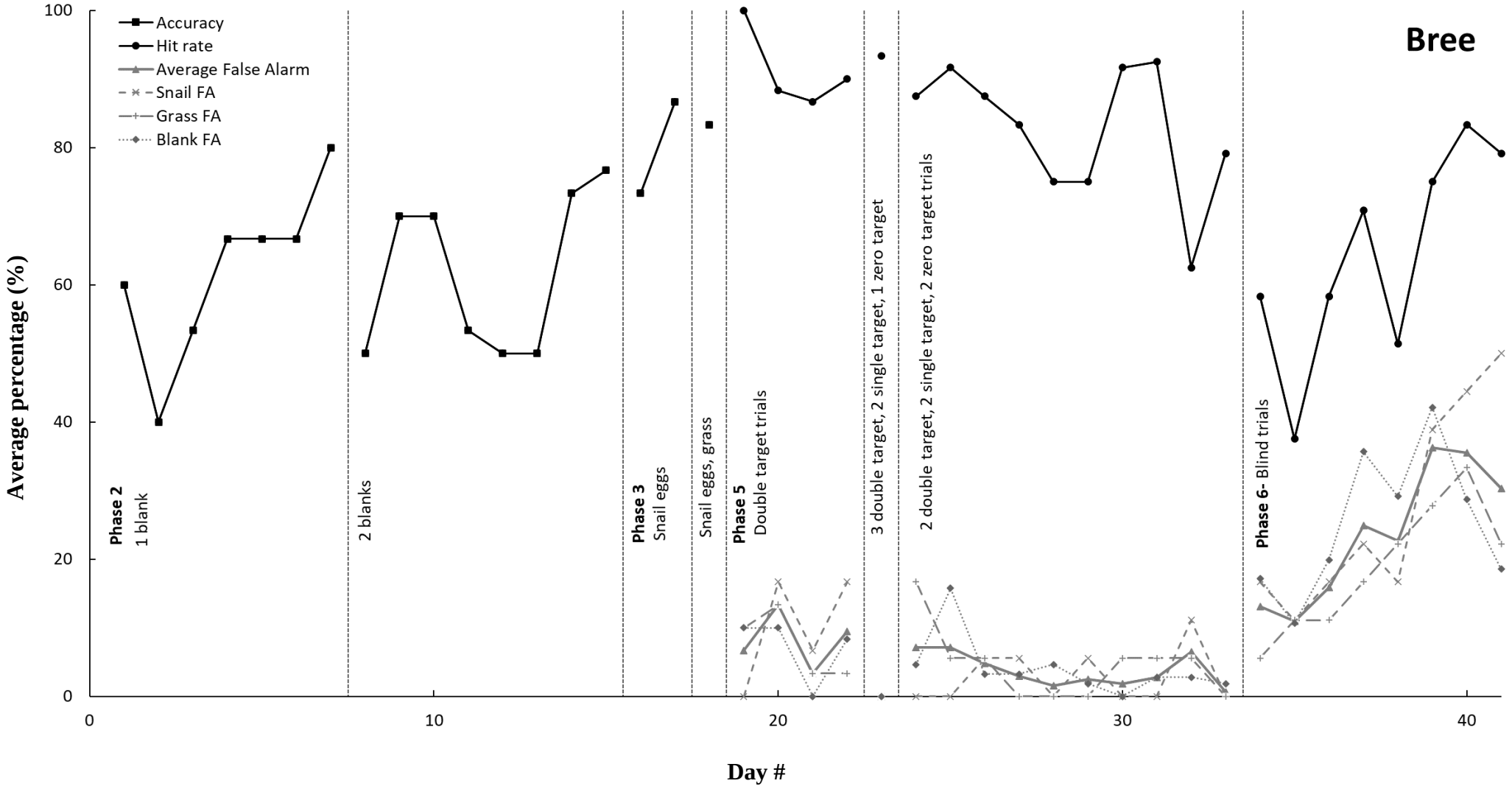

Figure 3.4 Bree's daily average accuracy (percentage of trials correct) in Phases 2 and 3 and daily average hit rate and false alarm rate across Phases 5 to 6, including false alarm rate for each sample type

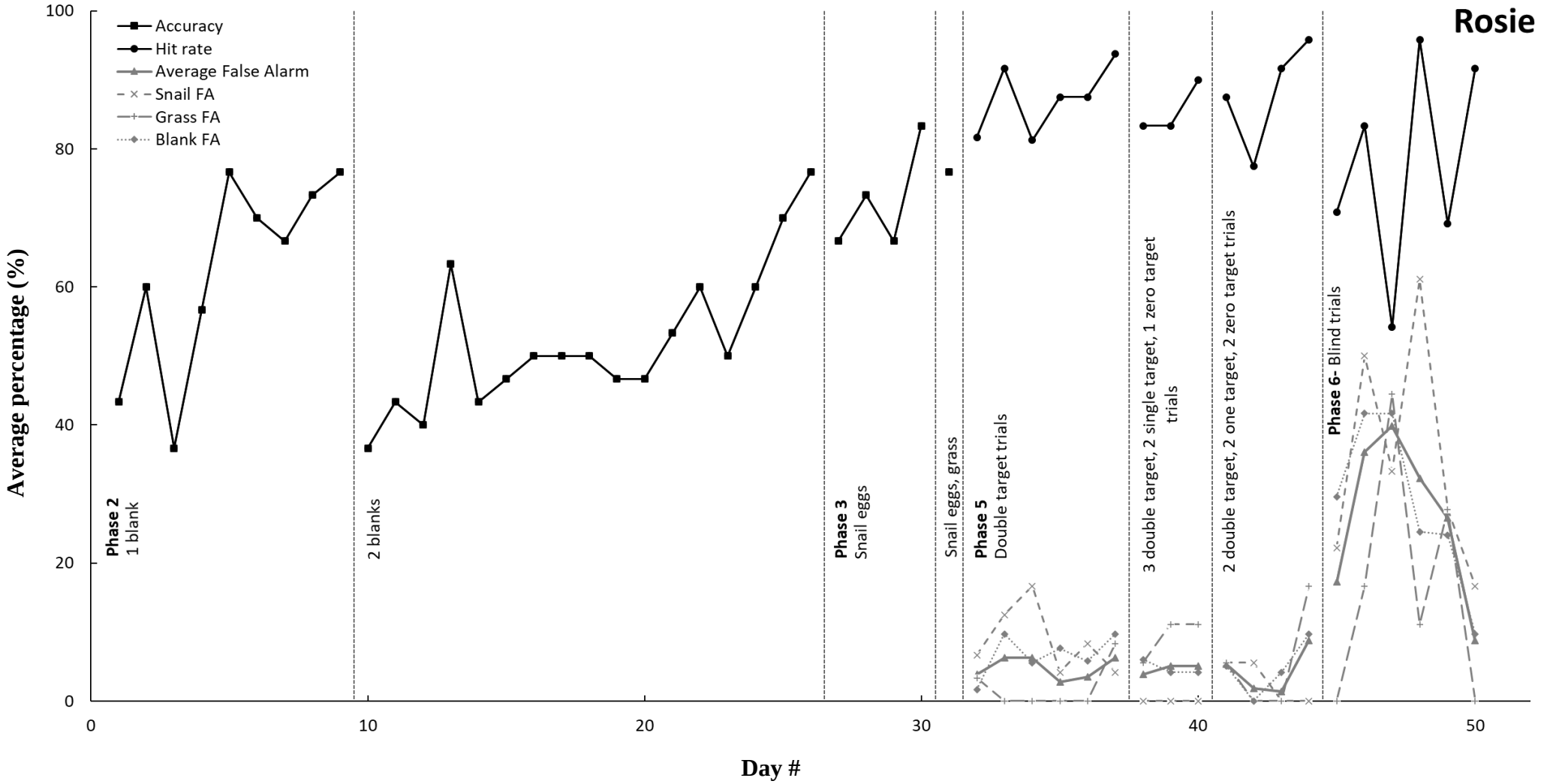

Figure 3.5 Rosie's daily average accuracy (percentage of trials correct) in Phases 2 and 3 and daily average hit rate and false alarm rate across Phases 5 to 6, including false alarm rate for each sample type

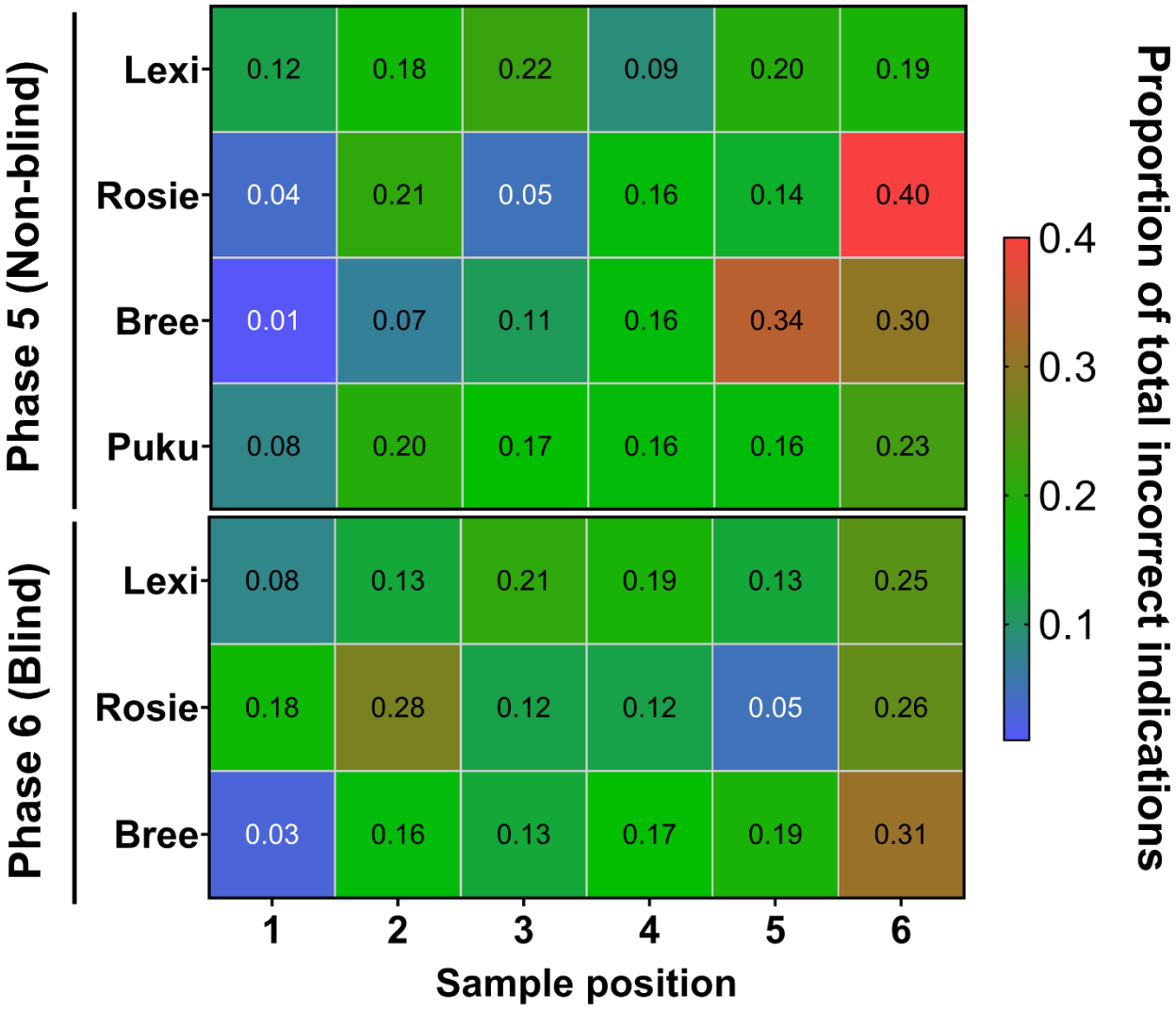

Figure 3.6 Proportion of total false alarm responses at each sample position for each dog in Phase 5 (non-blind trials) and Phase 6 (blind trials).

Chapter 4
Discussion

The objective of this study was to investigate dogs' ability to locate and discriminate whitebait eggs from non-target scents, with the aim of transitioning from laboratory to field conditions. This appears to be one of the first studies to investigate the use of dogs for this purpose; previous research by Dennis (2021) served as a starting point to this study. It was found that dogs (n = 4) could actively find and discriminate giant kōkopu eggs with a high level of accuracy (≥90% hit rate, ≤10% false alarm rate). The dogs performed well in increasingly field-like conditions, which included sessions where the target was present and absent, distractor odours were present (blank, grass, snail eggs), the experimenter was blinded to sample status, and the sessions were conducted outdoors.

4.1 Preliminary training stage

All five dogs participating in the preliminary stages (Phases 1-2) of the experiment were able to discriminate whitebait egg samples from blank samples. This was expected as only simple discrimination between the presence and absence of the target scent was required. During the first part of this stage, the ratio of target to non-target samples was 1:1, before moving to 1:2 once the progression criterion was met by each dog; similar to training conducted in other studies (e.g., Rosell *et al*., 2020). For the most part, dogs required more sessions to meet progression criteria when two blank samples were introduced, than when only one positive and one blank sample were presented likely due to the probability of selecting the correct sample reducing from 50% to 33.3%. Dogs were able to meet the criteria for this phase within 90 sessions across 30 training days (minimum: 14 training days/42 sessions, maximum: 30 training days/90 sessions). The results from this phase indicated that the dogs were able to discriminate the target scent from the no-scent (blank) samples and were a strong indication that scent learning was occurring.

4.2 Discrimination training

The aim of this stage (Phase 3) was to ensure discrimination of the whitebait eggs was occurring when dogs were presented with distractor scents. This was in line with the methodology of Rosell *et al*. (2020); however, instead of using strong distractor odours such as tobacco or coffee, scents that were likely to be encountered in field searches were chosen (i.e., grass or snail eggs). Four of the five dogs met the criteria to progress from this phase. The fifth dog was unable to meet progression criteria once snail egg samples were introduced, displaying long latencies (up to 3 minutes) before initiating sample investigation. Based on the dog's poor accuracy, it appeared that the dog had not achieved the

required scent learning. In contrast to the progression of the other dogs beyond this phase, it was decided that they be excluded from further trials.

Progression from the addition of snail eggs to the addition of grass samples occurred within 1-4 training days (3-12 sessions), and progression from the grass samples to the subsequent phase occurred within 1 day for each of the remaining four dogs. Dogs were able to meet criteria for this phase in 15 sessions across 5 training days (minimum: 2 training days/6 sessions, maximum: 5 training days/15 sessions). This was a relatively quick progression compared to the previous phase and suggests that the dogs had learnt the target scent and were demonstrating scent discrimination from the high correct indication rates, even after non-target scents were introduced.

4.3 Search pattern training

During this phase of the study (Phase 4) the aim was to train the dogs to systematically search each sample in the line-up. Not only would this ensure that the dogs were investigating each scent, but establishment of a systematic search pattern would be beneficial in a field environment, where the dogs would have to search along riverbanks. Lexi and Puku progressed from this phase in 9 and 21 sessions respectively, while Rosie required 45 sessions before meeting criteria. Rosie took longer to learn the pattern and would not actively "sniff" each sample. Instead, she would move directly to the positive sample at the end position and indicate. She required 39 sessions with the positive in the same position before she demonstrated systematic active sniffing of each sample. Once the positive position was randomised, Rosie's accuracy dropped in the first 3 sessions, requiring handler prompting to find the positive sample. Due to the amount of time spent in the previous stage, it appeared that Rosie had learnt to associate indicating at the end position with a reward and was no longer indicating based on scent. However, after a further 3 sessions with the positive position randomised, Rosie learned to indicate on the target and progressed to the next phase. The other dogs in the study learnt the search pattern relatively quickly compared to Rosie, progressing from this phase within 24 sessions across eight training days (minimum: 9 sessions/3 days; maximum: 24 sessions/8 days). Previous studies such as Rosell *et al*., (2020) and Wasser *et al*. (2004) established search pattern from the beginning of training, and it is unclear as to whether training scent discrimination or search pattern first is the optimal method of training scent-detection dogs. Having the dogs learn the search pattern with the handler present, whilst maintaining a high positive sample identification accuracy, was important for preparing the dogs for a transition into a field environment.

4.4 Assessment of scent discrimination

In the field, a positive find is not always guaranteed, or there may be multiple whitebait nests to be found in the search area. Therefore, it was important to present a varied number of positive samples

(Phase 5), as reliance on forced choice procedures may not provide accurate information regarding dog performance and scent learning, and these procedures do not always translate well into operational/field scenarios (Edwards *et al*., 2017).

Criteria to progress from this point required a hit rate of ≥90% and a false alarm rate of ≤10% to ensure confidence in the indications being made as phases moved towards field conditions. It was expected that there would be an increase in the dogs' false alarm responding in this phase, especially in trials with no positives present. However, false alarm responding remained relatively low (0-9.4%) across all the dogs, with the highest daily average false alarm rate being 8.8% from Rosie. By this point the dogs were maintaining a hit rate above 75%, but due to logistical delays, the dogs remained at this phase for maintenance training even when they met criteria to transition to the blind testing phase. Puku maintained progression criteria at this phase for five training days (15 sessions) before his hit rate performance became more variable, ranging from 54.1% to 95.8%. The drop in hit rate performance to 54.1% occurred three training days before training for all dogs was halted due to COVID-19 restrictions. Despite an improvement in hit rate performance before restrictions were imposed, Puku had not met progression criteria, and due to time constraints and the progression of the other dogs, Puku's participation was discontinued. Despite this, it is likely that Puku would have reached criteria again with sufficient time to conduct the necessary training. The results from this phase indicated that Bree, Rosie, and Lexi were independently discriminating the whitebait egg scent from the other scents, and this idea was strengthened as a result of the high accuracy rate that was maintained even when presence of the target scent was varied between trials.

4.5 Blind trials

The blind trials (Phase 6) were conducted to determine if the dogs' discrimination was controlled by the target odour, or by unintentional cues from the experimenter. Unintentional cueing can occur when unconscious reactions producing subtle changes in body language are interpreted by the dogs as clues (Johnen *et al*., 2017). It was essential that the handler be present during the line-up trials, and moving alongside the dogs while they worked, as this would occur under operational conditions. However, handler presence during training provides a myriad of potential challenges, as dogs are known to be expert readers of body language (Johnen *et al*., 2017). Dogs have been shown to be receptive to attentional states, and to understand and respond to visual cues as subtle as gaze and head orientation (e.g., Schwab & Huber, 2006; Soproni *et al*., 2001). Because the preliminary training and the indication response initially involved training with physical gestures from the handler, it is possible that the dogs were also conditioned to respond to unintentional cues given by the handler (Lit *et al*., 2011). It was therefore important to establish if unintentional cues were being used by the

dogs to identify the positive sample or if the dogs were discriminating on the basis of scent alone. Blind trials are an effective way of making this determination and validating the results gathered in the previous phases.

The increase of false alarm rates in the blind trials by the dogs suggests that there was some unintentional handler cueing occurring in the previous trials. However, following implementation of blind trials there was a decreasing trend in false alarm rates (Figures 3.2, 3.4, 3.5). Blind testing is commonly used in scent-detection studies (e.g., Oldenburg *et al*., 2016) and can be employed to determine if further scent discrimination training is needed. The effects of unintentional cueing were most prominent in Bree's false alarm rates, which increased notably in the blind trials compared to the previous phases, reaching 36% at its peak. Along with the increase in false alarm rate, hit rate dropped considerably for both Bree (37.5% at the lowest) and Rosie (54.1% at the lowest). This indicated that both dogs were relying, at least in part, on handler cues to determine which samples to indicate on. Rosie subsequently became less reliant on handler cues and was working independently enough to meet progression criteria in this phase, with Bree beginning to show promise of doing the same by the end of the study. This suggests that the blind trials were successful as a training tool to encourage independent searching by the dogs with less reliance on the handler cues. The blind trials also function to assist the handler in relying on behaviour from the dog rather than preconceived knowledge to locate the positive samples (DeMatteo *et al*., 2019; Lit *et al*., 2011). The results of the blind trials strongly indicate that Rosie and Lexi were independently discriminating the target scent without the influence of the handler and provide confidence that the dogs were demonstrating proficiency at detecting the target odour, offering potential for dogs to be used in the application of whitebait egg detection.

4.6 Outside trials

Progression to outdoor trials (Phase 7) introduced variability in factors that are typically controlled in a laboratory setting (e.g., temperature, humidity, wind, outside distractions) (DeMatteo *et al*., 2019). Because of this variability, it was decided that keeping aspects of the trial set-up the same as the indoor set-up would be the best way of introducing the dog to working in an outdoor setting (i.e., same trial arrangement, same sample presentation). However, progressing the trials towards an approximation of the field environment where the dogs would be working is important for training a successful scent-detection dog (Johnen *et al*., 2017). The movement to sample presentation in small jars was the first step towards approximating field conditions, as the jars were much more difficult to see in the grass, and Lexi required more time at this stage than she did in the blind trials and the initial stage of this phase. It was reasoned that making the samples difficult to identify visually from a

distance (Figure 2.8 (b)) would encourage more active sniffing and searching by Lexi, and initially she required verbal and gesturing prompts whilst moving down the line-up before working independently and meeting success criteria. The results of this phase indicates the potential that scent-detection dogs may have in a field setting, as Lexi was able to maintain a high accuracy and low false alarm rate within increasingly field-like settings, even when sample location was difficult to identify, further illustrating the potential of scent-detection for whitebait nest detection.

4.7 Position bias

It became apparent in the discrimination phases (Phases 5 & 6) that some of the dogs had developed position biases with regards to where they indicated. This is not uncommon in scent line-up studies and is typically mitigated through the randomisation of line-up order (Johnen *et al*., 2017). Position bias may have arisen due to training in the previous phases where positive sample location would remain in the same position until dogs met progression criteria. The position bias was less prominent in the blind trials where handler feedback was limited (Figure 3.6). The end (6^{th}) position bias likely originated from the search pattern training phase, where sample position was initially not randomised, and the positive sample remained at the 6^{th} position until success criteria were achieved. This bias was likely further cemented when an additional positive was added into line-up trials in Phase 5, and initially remained at the end position to encourage continued searching down the entire line of samples, before randomisation of all samples in the line-up. This suggests that the dogs were indicating on a specific position rather than a specific sample, based on memory of receiving reinforcement for the sample being in that position in previous trials (Johnen *et al*., 2015; Johnen *et al*., 2017). By the end of the blind trials, it appeared that Rosie had learned to indicate on the target scent and correctly reject negative samples in the end position by the decrease in false alarm responding, and Bree also showed signs of similar learning, which may have occurred due to the lack of feedback for incorrectly indicating on specific positions.

4.8 Implications

The results from this investigation support the prospect of using scent-detection dogs in whitebait conservation, with current survey methods for whitebait spawning sites being labour intensive and time consuming (Orchard & Schiel, 2021). As it stands, there is little knowledge of whitebait spawning sites, with sites usually being found at the interface of salt and freshwater, but whitebait species do not always return to the same locations to spawn, making predictions of nest locations hard to establish (Hickford & Schiel, 2011*a*; Orchard & Schiel, 2021). Previous studies have demonstrated that using conservation scent-detection dogs allows for a much larger search area to be covered in significantly less time than manual surveys (DeMatteo *et al*., 2019; Wasser *et al*., 2004. The ability

to locate more spawning sites will facilitate greater research and understanding of whitebait life history and their requirements for spawning habitat, providing vital information for the conservation of whitebait species (Orchard & Schiel, 2021). In addition, whitebait eggs can be morphologically similar to non-target species such as slugs and snails. The dogs used in this study demonstrated the ability to discriminate giant kōkopu eggs from non-target scents (snail eggs, grass) in increasingly field-like settings with a high level of accuracy (≥90% HR, ≤10% FA). This indicates that detection of eggs via scent may provide a more rapid method of identifying eggs than visual identification alone, providing an initial line of screening and reducing the number of samples that need more costly investigation such as DNA analysis (e.g., Franklin *et al*., 2015). Suggesting that application of scent-detection dogs for this purpose could provide an accurate, quick and comparatively cost-effective method for the detection of whitebait eggs and be a highly effective tool for the conservation of culturally appreciated whitebait species.

4.9 Limitations

Delays in training and testing due to COVID-19 lockdowns and logistical support resulted in performance changes from the dogs. Without these delays, it is possible that more dogs would have met progression criteria in blind trials and advanced to outdoor training. Sample line-ups are labour intensive, especially when there are multiple rearrangements of samples per session. Delays associated with rearranging samples appeared to impact the performance of some of the dogs, as sessions could take over 15 minutes to complete. To mitigate this, sessions were reduced from 10 trials to 6, which reduced the amount of time per session. However, once the trials moved outdoors, because of the longer inter-trial intervals session time was increased due to the complexity of an outdoor trial. From observations in this study, it is recommended that trial time be reduced to less than 10 minutes to prevent fatigue and loss of motivation by the dogs.

Samples were thawed and re-used multiple times throughout the study. This is a practice often implemented in scent detection research for several practical reasons, such as limited availability of target samples (e.g., Grimm-Seyfarth *et al*., 2019; Kerley & Salkina, 2007). Samples were re-used between dogs which allows for potential scent cueing, with the dogs possibly detecting scent of the other dogs and not necessarily that of the whitebait eggs. Whitebait eggs were refreshed halfway through the study and fresh eggs were used for the outdoor trials to mitigate this, and it is notable that there was no appreciable difference in the dogs' performance following the refreshing of these eggs. However, due to issues procuring snail eggs, the same eggs were used for the duration of the study. This may have resulted in odour decay over time, potentially modifying the scent profile of the snail eggs (Parsons *et al*., 2018).

There was potential for cross contamination of odours, as the odours released from the samples were not confined to containers but left open whilst in the line-up. This may have resulted in odour movement throughout the room, increasing the difficulty of recognising and locating the target scent within the line-up. Wearing and changing of gloves when interacting with samples, proper cleaning of equipment, and the covering of samples with the metal colanders were implemented to help prevent any cross contamination. However, in the outdoor phase, minimisation of scent contamination with other odours from the field was difficult even with gloves due to samples being placed by hand into the grass. However, this may have acted as an additional training aid, as in the field dogs would have to detect the eggs amongst an array of other scents.

4.10 Future research

Although the findings of this study demonstrated that dogs could discriminate whitebait eggs from non-target scents in progressively field-like conditions, completion of the transition to a field setting and operational testing of the dogs is still required. Determination that the whitebait egg scent can be detected by dogs amongst various background smells in field settings must be confirmed before efficacy can be assured. Further work also needs to be undertaken to confirm scent generalisation across the five whitebait species, and if generalization does occur, then dogs could be used to detect any of the whitebait species without additional training. If generalization does not occur, further research could investigate what types of training would be required for scent generalization to occur.

Potentially, dogs could be trained to detect rarer and more endangered species such as shortjaw kōkopu, where current knowledge on spawning sites is limited (Yungnickel *et al*., 2020). Future work could improve the training process, possibly investigating the use of different scents in the line-up. For example, the addition of different fish egg scents (e.g., cyprinid eggs) that may be found in whitebait habitats may help mitigate scent misperception or false indications if encountered in the field. Because training, maintenance and welfare costs associated with use of scent-detection dogs can prohibit their use (Rutter *et al*., 2021), recruitment of volunteer dog and owner teams could be explored. Teams could be implemented in periods when whitebait eggs are laid and used to mark and protect future spawning sites. Training programs and maintenance could be undertaken in conjunction with already successfully implemented teams and could prove to be extremely successful and cost effective, as demonstrated in other studies examining the benefits of using pre-existing dog-handler teams to assist training volunteers and their companion dogs to detect novel odours (e.g., Rutter *et al*., 2021).

There is also potential for dogs to be trained to detect spawning sites of invasive fish and other animals, helping to eradicate them from waterways. Successful detection of common carp spawning sites in

Lake Crescent, Australia, has assisted in maintaining an invasive fish free environment, using netting and exclusion of the fish from spawning habitats (Taylor *et al*., 2012; Yick *et al*., 2021). Using dogs as a quick, non-invasive detection method for spawning sites could allow for large-scale eradication operations from waterways nationwide.

4.11 Conclusion

Whitebait species make up an extremely important cultural, recreational, and commercial fishery in New Zealand (Fyfe & Bradshaw, 2020; Wylie *et al*., 2016). However, they are declining due to several anthropogenic factors (Hickford & Schiel, 2011*b*). Detection and protection of spawning sites is essential to preserving these species, but finding these sites is difficult due to nest habitats being located amongst dense riparian vegetation and whitebait species not always returning to the same locations to spawn (Orchard & Schiel, 2021). Current spawning site identification survey methods are labour intensive and time consuming (Orchard & Schiel 2021), and conservation scent-detection dogs have previously demonstrated usefulness at providing quick and accurate target identification, within much larger search areas than manual surveys alone (DeMatteo *et al*., 2019), suggesting the potential of dogs for this application. The results of this study indicate that dogs have the ability to find and discriminate giant kōkopu eggs from several other non-target scents, which was validated by the success of the blind trials. The encouraging outcomes of the outdoor trials illustrate the potential of using dogs for whitebait spawning site detection, which could be further investigated through continued progression into field environments. The results of this study suggest that scent-detection dogs could be a valuable conservation tool for the detection of whitebait eggs in field environments, possibly providing a cost effective, accurate, and time saving method compared to visual search methods that are currently employed.

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Appendices

Appendix A: Handling and care of pet dogs for research

General:

This document outlines the general procedure for the handling and care of pet dogs being used in research. This protocol has been developed with reference to the Animal Welfare (Dogs) Code of Welfare (Animal Welfare Advisory Committee, 2010, http://www.mpi.govt.nz/protection-and-response/animal-welfare/codes-of-welfare/)

Location/Equipment:

- Dog studies will be carried out in the TTH1 and TTH4 houses on the campus of the University of Waikato (on Ruakura road next to gate 3). TTH1 has 5 separate rooms; one room for the apparatus to run the experiment, two rooms with kennels to house the dogs, and other rooms used for sample preparation and working space. TTH4 has a similar arrangement but can be used as two separate canine research facilities (with separate kennel and experimental spaces). Adjacent to the experimental facilities is a grassy field in which the dogs will be exercised and allowed to toilet while on leash.
- A maximum of 5 dogs will be present in any of the facilities at any one time.
- Dogs will not be held at the facility overnight. Arrangements will be made by the researcher to meet owners when they drop off their dog before each research session and for pickup afterwards. Under exceptional circumstances, after consultation with supervisors, a researcher may pick up or drop off the dog from the dog owner's home.
- Each dog will be held in a separate kennel, crate, or tie-up station containing bedding, toys, and a bowl of fresh water.
- A logbook will be kept with the name of the dog, arrival time and collection time, and contact details of owner. Dog's owner will sign in upon arrival and sign out when they have picked up their dogs.
- Dogs must wear a collar and be on a lead at all times, except when they are in the kennels or in some cases in the experimental room (depending on the exact requirements of the particular study).
- All dogs must be fully vaccinated up to date for the core diseases: distemper, hepatitis, parvovirus, and leptospirosis. Kennel cough vaccination is also required. Vaccinations are confirmed by sighting and photographing the vaccination certificate; all vaccination records will be stored in a database and reviewed monthly – if a dog's vaccination is due, the owner will be asked to bring the dog's updated vaccination record into the facility and the photograph/record updated accordingly.
- All food will be stored in labelled sealed containers within the storage room.

Record Keeping:

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

Cleaning:

- Dog pens and the experimental room will be vacuumed and soiled surfaces will be cleaned with disinfectant at the end of each day after dogs have left.
- If any faeces or urine is deposited inside the facility, they will be disposed of appropriately and cleaned thoroughly. For example, disposable gloves will be worn, and a plastic scoop will be used to remove any faeces which will then be bagged and disposed of in an external rubbish bin outside of the dog facility. The area will then be cleaned thoroughly using an appropriate cleaner.

- A foot pedal rubbish bin will be used to contain general waste within the dog facility, and this will be emptied as appropriate.
- Bowls will be washed thoroughly each day with a disinfectant that is suitable for food surfaces that kills both viruses and bacteria and then rinsed.
- Pest control for insects and rodents will be applied as necessary. These control methods will be used in such a way that dogs cannot access them. If there is any risk of dogs accessing them, a non-toxic (to dogs) control method must be used.

Animal Handling/General Care:

- One person will be in attendance when dogs are present at the laboratory. Another person will be aware and available on call during the running days.
- Before removing a dog from its kennel, external doors to the facility will be closed, and the dog must be put on a lead.
- Dogs will be taken out to walk and toilet every 2 hours, and dog waste disposed of appropriately. Volunteer dog walkers who have been recruited and trained according to Appendix 2 may be used to walk dogs involved in research at the University of Waikato.
- Dog walkers will take care to prevent dogs from accessing bait stations that are positioned around campus. An up-to-date map of the location of these stations will be posted at the exit of each building. Walkers will also prevent dogs from accessing discarded food/trash and, if there is any indication that they have eaten something inappropriate, they will immediately report this to the lab supervisor.
- Any dog showing signs of aggression towards the researcher will cease participation in the study.
- Any dogs showing persistent signs of distress or fear will cease participation in the research and the owner will be contacted.
- Dogs that are transported in vehicles by researchers will always be transported in a manner that is safe and approved by the dog's owners. Dogs will only be transported individually, with the exception of dogs that live together.
- If a dog becomes ill/injured, the laboratory manager will call the owner's vet and take the dog to the vet immediately. If the owner's vet is unavailable, the dog will be taken to the local vet (Newstead Vet) or the after-hours clinic if the local vet is closed. The owner will be contacted as soon as practical. A vehicle must be available (i.e., on campus) for emergency transportation at all times when dogs are present at the facility. If the dog's illness/injury occurred as a result of involvement in the research project, the School of Psychology will pay for the veterinary services. Otherwise, the owner will pay for the services.
- Multiple dogs will only be present at the facility at the same time if the owners give permission for this to occur, and if they state that their dog is friendly towards other dogs (as part of the consent form). The dogs will be kept on leads around each other and held in separate kennels/crates/tie up stations (unless they are from the same household and the owner prefers them held together). Only dogs that are confirmed to be reliably friendly toward each other (including those who live together) will be walked together. If any conflict behaviour is seen between dogs, then the dogs will be separated from each other and the senior researcher will be contacted.

Building Security:

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

Emergency Evacuation Procedures:

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

Versions and Reviews: Version 2.1
Date revised: October 2019
Date approved: 18 October 2019
Next revision due: 19 October 2022

Appendix B: Consent form

CONSENT FORM
Researcher's Copy

THE UNIVERSITY OF WAIKATO
Te Whare Wānanga o Waikato

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

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

PRINCIPAL INVESTIGATORS

Ashlee Cooper, ashleejanecooper@gmail.com
Dr Tim Edwards, 07 837 9409, tim.edwards@waikato.ac.nz
Dr Clare Browne, 07 837 9394, clare.browne@waikato.ac.nz

PURPOSE OF THE PROJECT

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

DESCRIPTION OF PROCEDURE

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

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

COSTS TO OWNER

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

WITHDRAWING MY PET FROM THE PROJECT

I understand that participation in this project is entirely voluntary and that I may withdraw my pet at any time without any negative consequences. I understand that my dog might be withdrawn from the project if a vet finds it is necessary and in my dog's best interest.
If I have additional questions regarding this project, I may phone or email the principal investigators.

ADDITIONALLY

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

AUTHORISATION

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

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

Yes / No

My dog is friendly towards other dogs:

Yes / No

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

Yes / No

Pet's name: _____

Owner's name: _____

Owner's signature: _____ Date: _____

Researcher's signature: _____ Date: _____

Appendix C: Scent detection project dog recruitment standard operating procedure

1. Purpose

This standard operating procedure (SOP) provides guidelines and standardised procedures to be adopted when recruiting dogs for scent detection projects at the University of Waikato Hamilton campus.

2. Recruitment

1. Dogs may be recruited by talking to other dog owners encountered on walks around the university campus, by handing out fliers in previously agreed locations or posting to social media platforms approved by the project supervisors/university.
2. All initial contact with the project will be established via the official dog lab email address: dogs@waikato.ac.nz managed by Dr Clare Browne.
3. Clare will refer the message to the researcher in charge of recruitment at that time.
4. A preliminary email (see model in Owner SOP folder) from the researcher will be sent to the prospective owner, including the three consent forms (also in folder) required to enrol a dog in the programme.
5. When the consent forms have been filled out and returned, the person running that project will reply to the owner via email with the offer of an initial meeting at the laboratory (see model in folder).

3. Initial interview

1. Children are not allowed at the lab. However, owners are welcome to bring any other interested adult who might conceivably end up picking up or dropping off the dog.
2. The researcher will introduce themselves and explain their role.
3. They will provide the prospective owner with details about their project, other people involved and the requirements (e.g.,, the level of commitment involved).
4. During the preliminary meeting the dog is off-lead in the workroom.
5. The owner and researcher have a chance to observe the dog's behaviour.
    1. The dog will be briefly crated or penned.
    2. They will separated from their owner for a short period of time (The owners stand in the vestibule for around 20 seconds).
    3. The dog's level of arousal and their responsiveness to kibble (or other food if applicable) during the meeting will be a matter of on-going discussion.
6. A weekly timetable form will be filled out by the owner (see folder). A model will be provided, as some of our owners find this task difficult.
7. After 30 minutes there should be consensus between the owners and the researcher about whether or not to proceed with training.

4. Once on a project

1. The dog's gear should be inventoried. Photograph any gear that arrives with a dog, with the dog so there is no confusion about whose stuff is what. Do not give one dog's gear to another dog.

1. If the dog wears a harness, photograph the dog wearing it, and ask the owner to supervise you putting it on, at least once to ensure that you will be putting the gear on correctly when alone.
2. Take a picture of the dog in their working dog jacket for the owner.
3. Any factors that relate to a dog's presence on the programme or their general wellbeing need to be mentioned to owners on an ongoing basis.
4. Tell the owner about every advance and milestone the dog achieves. Showing some footage during pick up may be a good idea.
5. Specific details about activities, performance criteria and the dog's performance should be used to cue the owner about the suitability of the dog. **No owner should be surprised if their dog is being dropped from the programme.** Provide a 'countdown' timeframe ('one more session to reach criterion') and specific details ('She is still scared of the feeder; she sits behind my feet and will not approach it. She barks at the sound of it dispensing food, and won't approach the feeder to eat.') as soon as you become concerned that the dog may not be a good fit.
6. Remember that an excellent family pet may not necessarily be a good research dog. It is no reflection on the dog or the owner. Dogs have actually been dropped from the programme for being reluctant to do anything in the workroom unprompted. This would be desirable behaviour in a number of different contexts, just not at the dog lab.
7. Incidents need to be reported immediately to owners on pickup or via phone during the day if sufficiently serious. (Examples of 'phone contact issues' include but are not limited to 'your dog is being aggressive to all of the other dogs and/or me', 'your dog is ill', 'your dog has been highly agitated and is not settling after a range of strategies including penning in the office etc have been used', 'your dog has tried to attack the crate and is at risk of hurting themselves'.)
8. Supervisors need to be alerted to serious incidents, and be given an opportunity to support you and the owner at handover if required.
9. In extreme cases, an incident report may need to be written, describing who, when, what, where and the outcome.

Appendix D: Standard operating procedure for training dogs to detect whitebait eggs

NOTE: Procedures for dog selection, habituation, handling, and care have been omitted, as requirements are likely to vary among laboratories. The complete standard operating procedures specific to the author's laboratory are available upon request.

Experimental Laboratory Setup

Position samples in a way that can be easily viewed by cameras and so the handler may stand at one end of the line-up while the dog is working in a direction that faces away from the handler. Handler needs to have a clear view of samples and dog position within the room, to allow for prompt reinforcement of positive indications.

Training

Phase 1. Introduction

Dog must be familiarised to the researcher and to the operating room before training can begin. Dog should be allowed to investigate the operating room independently, and the researcher should take time to allow the dog to investigate their scent and get used to their presence. Verbal and food reinforcement may be given at this time to help form a positive relationship between the researcher and the dog. Allow dog to investigate the surroundings, until the dog is visibly more relaxed in the environment. Once the dog is habituated to the environment and researchers, training sessions can begin- look for signs of relaxed body language from the dog and that they are attentive towards researcher. During the shaping and throughout the training process, the session should be terminated if the dog is showing signs of fatigue, stress, or disinterest. Sessions should aim to finish immediately following a correct response and reinforcement.

Phase 2. Shaping Sniff and Indication Response

Beginning with a single positive sample in the room, the handler and dog enter the experimental room. Handler stands against a wall facing the sample and gives the command of "go find". The dog is rewarded with the handler giving verbal praise and feeding the dog a treat by hand for approaching, interacting, or sniffing the sample (depending on what occurs first). In the initial training phases, the dog should be reinforced for any movement toward or with the sample, eventually being reinforced only for sniffing or appearing to sniff the sample. If the dog needs prompting to interact with the sample, the handler may need to point or approach the sample, whilst giving the "go find" command to encourage investigation of the sample by the dog.

A reliable sniff of the sample is determined as showing interest and touching nose onto the stainless steel colander or inside the steel cake tin (within 2 cm) that the sample is held in for 5 out of 6 trials. Once the dog is reliably sniffing the sample, the positive indication response of a sit-stay can be shaped. Upon the dog sniffing the positive sample, the handler will give the instruction of sit and will reinforce with praise and a treat when the dog performs the sit behaviour. When the dog is reliably sitting after interacting with the positive sample, a "stay" command will be applied, with the handler increasing the distance between themselves and the dog before reinforcement, where the handler will walk to the dog to reinforce. Once the dog is indicating with a sit and stay at the positive sample while the handler is in their

observation position, the "stay' command should be phased out accordingly and only used if necessary. The handler may then work on extending the stay duration, as may be needed in the field to ensure that the dog is reinforced for a correct response. Upon handler approaching the dog for indication reinforcement, the handler should extend the duration of the stay upon approach, starting at a 1s delay and working upwards to 20s, dog must remain in a sit stay position for 3 consecutive commands at each stay duration. Dog can progress to next phase if it is reliably indicating at the positive sample with a sit and stay up to 20s for 3 commands in a row.

Phase 3. Introduction of blank samples

Blank samples are introduced to ensure dog is trained to detect the scent of the positive sample. Begin with one positive and one blank sample laid out in the experimental room. Handler will bring dog into the room and the dog will be told to "sit" and "stay" in a starting position at one end of the room, adjacent to the samples. The handler will move against a wall facing the samples, ensuring that body language is neutral and eye contact is not on the dog, so not to cue the dog to the correct sample. The dog is told to "go find". Once dog indicates on the sample, it must remain in a sit position working from 1s up to 5s while the handler is looking at it, to encourage confident indication. Once appropriate indication duration is achieved, reinforcement of verbal praise and a treat will be given only when the dog indicates at the positive sample. If the dog indicates on a negative sample, it will be reset to the starting position and will move to the next trial. 10 "go find" commands are given per round and dogs undergo 3 rounds in one session. Dogs must receive a correct indication rate of 80% for 2 out of 3 rounds to progress to an additional sample. Sample position changes for each trial and is established using the RAND function on Microsoft Excel. Dog must remain in the starting position whilst the handler reorders samples, and the handler will move back to neutral position before instructing the dog to "go find" again. Once dog meets criteria of 80% correct responses for 2 out of 3 rounds, an additional sample can be added. Continue to increase blank samples up to 2 blanks to one positive, with the criteria of two out of three 80% correct rounds being met, before moving to distractor scents like snail eggs or debris. Trials expire if the dog does not reliably indicate on a sample after 20 seconds has elapsed and will be called back to the starting position and begin the next trial.

Phase 4. Discrimination and Distractors

Introduction of distractor samples to ensure scent discrimination from dog. Distractor samples may include snail eggs, and organic matter that may be encountered when in a field setting. A similar set up of samples should be adopted as in phase 4 of one positive and 2 blank samples, with the addition of 1 non-target sample. Samples should be laid out in a line, with the position of the positive sample and non-target being established using the RAND function on Excel. Dog is told to "go find" and must indicate only on the positive samples. Once the dog achieves an 80% correct indication rate for 2 out of 3 rounds an additional non-target sample of a different scent can be added. The same non-target samples are used each time, with the only change being the addition of another non-target once criteria is met.

Phase 5 . Training Search Pattern

Dogs to be trained to systematically search presented samples, to ensure that the dog is investigating all scents before making an indication. To begin with, the scent line-up will consist of the positive sample being at the position furthest away from the dog, as previously done by Rosell *et al*. (2020), to encourage investigation of all samples presented in the line-up. The rest of the line-up will consist of the already introduced blank and distractor scents (snail eggs, grass). The handler will be positioned in the starting position next to the dog and will give the

command to “go find”. Walking alongside the samples and the dog, the handler will prompt investigation of each scent in the line-up, by either using their presence or pointing/gesturing. If the dog indicates on the correct sample, it will be rewarded immediately with a treat and verbal praise. If the dog and handler get to the end of the line-up, the dog has the remainder of the trial time (20s) to make an indication response independently before the trial is reset. After one session round (10 trials), the dog’s systematic search performance will be evaluated. If the dog is not systematically investigating each sample in the line-up, then the dog will complete another 10 trials with the positive in the end position and handler prompting. If the dog is effectively and independently searching each sample presented, the position of the positive sample will be randomised using the RAND function on Excel. In the randomised positive position trials, the criteria to progress will be based on the dog systematically searching each sample and achieving an 80% correct indication rate for 2 out of 3 rounds. In the randomised trials, the dog is expected to stop and indicate once it reaches the positive sample, which may need to be prompted by the handler, to ensure that the dog is not going back and forth along samples in the line-up. The dog can investigate samples adjacent to the positive sample before making an indication, but if the dog moves further than the immediate adjacent samples, it is considered an incorrect indication and the trial will be reset. Once the dog indicates on the positive sample as it comes across it in the line-up, the dog is expected to sit and wait for up to 5 seconds before reinforcement is given. Once reinforced, the dog is brought back to the starting position and the next trial is begun.

Reference: Rosell, F., Kniha, D., & Haviar, M. (2020). Dogs can scent-match individual Eurasian beavers from their anal gland secretion. *Wildlife Biology*, *2020*(2).

Phase 6. Introduction of additional positives and randomisation of positive samples

In this phase, the addition of more positives will help to encourage the dog to continue working down the line to teach them that there may be more than one correct response when going into a field setting. The randomisation of positive sample amounts later will also help prepare them for a field setting where there may be more than one or no correct answers. To begin with, one positive will be positioned at the end position furthest away from the dog and the additional positive will be positioned randomly elsewhere in the line-up. The dog will begin at the starting position and be told to “go find”, once the dog sits and indicates on the first positive sample for 5s, it will be rewarded and told to “go find” from where it is positioned within the line-up. The dog will be rewarded for continuing down the line systematically and indicating on the additional positive sample at the end position. Indication and correct/incorrect responses for each sample position will be recorded and when the dog receives a daily average hit rate ≥90% and false alarm rate ≤10% the position of both positives will be randomised the next day using the RAND function on Excel. Dogs will complete 3 sessions of trials, and once meeting criteria, the number of positive samples should begin to be randomised to either 1, 2, or 0 positives in the line-up. To prevent discouragement of searching by the dog, the amount of all negative trials should be limited to two or less per session, with the rest of the trials consisting of either one or two positive samples. Positive sample number will be randomised using the RAND function on Excel and correct indication, false alarm, incorrect responses for each sample position will be recorded. For the varied positive trials, dogs will complete 3 sessions of 6 trials, to make sure that the dogs are not spending too much time in the experimental room. Once the dog receives a daily average hit rate ≥90% and false alarm rate ≤10%, it can move on to the next stage.

Phase 7. Single Blind Trials

Blind trials to be completed to ensure that dogs have learnt true scent discrimination and are not being cued by handlers as to the location of the positives. Dogs will participate in 3 sessions of 6 trials, with either one, two or zero positive samples per trial. Order of the line-up and placement of samples into the experimental room is to be determined by an assistant while the dog and handler wait outside of the room. The assistant will leave the room and will watch the trial using video footage and record dog response to samples in the line-up (either correct or incorrect indication, correct or incorrect response). Handler and dog will enter the room and the handler will give command for the dog to "go find". Upon indication by the dog, the handler will wait 5 seconds and then approach the dog and sample and check sample to see if a correct indication has been made, giving a reward for a correct indication and then encouraging the dog to continue down the line with another "go find" command. Once the dog has reached the end of the line-up, dog and handler will leave the room for the assistant to enter and arrange the next trial. Gloves will be worn by experimenter and handler when interacting with samples and will be changed between trials and after directly touching samples, when preparing the samples. When the dog receives a daily average hit rate ≥90% and false alarm rate ≤10%, it can move on to the next phase.

Phase 8. Transition to outside setting

Line-up to be transferred to an outside setting as a transition phase towards more field-like trials. Trials will follow the same principles as in Phase 6, with 3 sessions of 6 trials, consisting of two zero positive trials, two single positive trials and two double positive trials. Samples will be arranged at varying distances along a fence line or building, to help simulate the search pattern needed when running along riverbanks in field settings. The number of positive samples for each trial in the session will be randomised using the RAND function on Excel, as well as the order of the lineup for the snail, grass, blank and positives in each trial. Dogs will be secured to a hitching ring between trials and always kept on a leash during outdoor trials. Samples will be presented in the same metal bowl/cake pan setup as in indoor settings, which will be prepared and taken to the outdoor experimental area prior to bringing the dog outside. Gloves will be worn while preparing and presenting samples, as well as during trials, being changed regularly. A camera, tripod and hygrometer will be set up with a clear view of the trial area, and clipboard and datasheet will be hung somewhere that is easy for the experimenter to record data. The dog will be brought outside and attached to the hitching ring and the experimenter will record temperature and humidity, begin recording on the camera and proceed to arrange the samples as had been determined by the RAND function prior. Once ready, the experimenter will put the dog on a long lead, and the dog will be told to sit and stay at a starting point at one end of the line-up before being given the "go find" command, prompting down the line as needed. Once the trial is finished, the dog will be reattached to the hitching ring and the samples rearranged for the next trial. This will repeat until all 6 trials are completed, where the dog will have a short break before starting another session. Indication and correct/incorrect responses for each sample position will be recorded. Dog must receive a daily average hit rate ≥90% and false alarm rate ≤10%, it can move on to the next stage.

Once success criteria is met, trials will take place in the same format as the previous stage, but samples will be presented in small glass jars to encourage active sniffing, due to samples being less visually obvious. Samples will be transferred to small glass containers and covered with an aluminium lid drilled with 5 holes and placed in a line-up within the grass. Once ready, the experimenter will put the dog on a long lead, and the dog will be told to sit and stay at a starting point at one end of the line-up before being given the "go find" command, prompting down the line as needed. Once the trial is finished, the dog will be reattached to the hitching ring and the samples rearranged for the next trial. This will repeat until all 6 trials are

completed, where the dog will have a short break before starting another session. Indication and correct/incorrect responses for each sample position will be recorded. Dog must receive a daily average hit rate ≥90% and false alarm rate ≤10%, it can move on to the next stage.

In the event of bad weather, any outdoor trials will be transitioned back inside as a form of additional training for the dogs. Trials will be paused if distractions such as other dogs, noisy machinery, groundskeeping work etc. is being performed within or nearby the trial area and restarted again once the distraction has ended.

Appendix E: Video links of dogs working:

Indoor training trial- Phase 3: 5 samples (Bree)

See supplementary folder or:

https://drive.google.com/file/d/1I8RG5kFkY3OudGPcS03YrH834IeQRNp0/view?usp=sharing

Indoor blind trial- Phase 6 (Rosie):

See supplementary folder or:

https://drive.google.com/file/d/1a1BxNdXKsFw1S4BQKvYsFLLNHujeff7m/view?usp=sharing

Outdoor small jar trial- Phase 7 (Lexi):

See supplementary folder or:

https://drive.google.com/file/d/1Gynqbmsju20dnZhX2cyxxExu-WKy5OGp/view?usp=sharing