

**Using Conditioned Food Aversion (CFA) to reduce  
Pied Crow (*Corvus albus*) predation of plover nests**



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Minor dissertation submitted in partial fulfillment  
of the requirements for the degree of  
Master of Science in Conservation Biology

February 2016

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## PLAGIARISM DECLARATION

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Date: 15<sup>th</sup> February 2016

## **ACKNOWLEDGEMENTS**

I am sincerely grateful to numerous people for dedicating time and effort to this project, and to those who made it possible for me to do this course. First and foremost I must thank my supervisors, Dr Thomas Flower and Dr Robert Thomson, for your unwavering support and encouragement. To Tom, for keeping up communications and always being ten steps ahead of me despite being ten hours behind in Canada. I greatly appreciate your guidance, insight, patience and enthusiasm throughout all components of the project - "Jolly good, push ahead". To Robert, for always being available to chat through aspects of the project, for making sure that I was surviving fieldwork in Velddrif, and most of all for regularly reassuring me that, "It'll be fine." You were right, but you and Tom played a huge role in making that happen.

Special thanks go to Professor Peter Ryan, Dr Susan Cunningham, Professor David Cummings and Hilary Buchanan for being so understanding at the start of the course. I cannot thank you enough for being so accommodating and supportive in those first few weeks and for facilitating me to keep up with coursework from afar. Additional thanks go to Dr Robert Little and the Percy FitzPatrick Institute of African Ornithology for granting me the Gordon Spriggs Scholarship, as well as to Anthea Links and Tania Jansen for your administrative support during fieldwork.

I extend my thanks to others involved with the fieldwork component. To those who granted permission for me to conduct experiments on their property: Steve Lombard (Cerebos), Joan and Yntze Shrauwen and Timothy Mayes (Velddrif Salt Company), Jan and Malani Kotze (Kuifkopvisvanger) and Jurgen and Mariette Kotze (Kliphoek Farm). Special thanks go to Jan and Malani for being such great hosts and Jan especially for being a helpful nest-finder and for rescuing me from the mud and sand on several occasions. To Jolyon Troscianko, Jared Wilson-Aggarwal and Claire Spottiswoode for providing GPS coordinates of previous nest locations and for giving me valuable information from your research in 2013. Selengemurun Dembereldagva and Rachel Shaw helped with egg preparation for which I am immensely grateful. Two dear friends, Gemma Walker and Andrew deBlocq made fantastic research assistants. Thank you for making days in the field and evenings at the caravan full of fun and laughter. I am deeply

grateful to my Velddrif mom and dad, Linda and Eddie du Plessis, for welcoming me into their hearts and home, for taking care of me and giving me a greater appreciation for the incredible birdlife in the area.

I am immensely grateful to my family; John and Deborah Anderson, Douglas and Chloë Coltart, and all those who contributed financially to making this year possible. It has been a tremendous honour to do this course, a dream that began during my first year of undergrad and you helped make it a reality. Thank you to my mom and dad, Sally and Gordon Ferguson, who have supported me through thick and thin to get me to where I am today. I could not have made it this far without your steadfast love, support and encouragement. To my friends and family who have supported me throughout my university career and especially in the year gone by - thank you so much. Above all, thank you to my Father in heaven, for giving me a passion for conservation, the ability to learn and provision in all things.

Last but by no means least, to the Conservation Biology Masters Class of 2015/2016; Penny Pistorius, Jessleena Suri, Julia Van Velden, Adele Cormac, Gabriela Fleury, Jennifer Angoh, Elke Visser, Christiaan Willem Brink, Kyle Lloyd, Tokura Wataru and Hermenegildo Matimele. We survived 13 months on the CB island. Words cannot express how grateful I am for the friendship of each and every one of you. Thanks for running this hurdle race with me, all the way to the finish. I wish you all the very best in the future and look forward to working alongside you as we work for the greater good of our natural world.

## ABSTRACT

Nest predation is one of the principle constraints on bird breeding success, accounting for 20 to 80% of all nest failures. It can be exacerbated by anthropogenic factors and the resultant increased predation pressure has caused the decline of numerous bird species worldwide. Identifying management strategies to reduce nest predation is consequently a priority for biodiversity conservation. Many lethal and non-lethal methods of predator control can be ineffective, unethical, time-consuming and expensive to implement. An alternative is the use of Conditioned Food Aversion (CFA), a method by which animals are deliberately induced to avoid nests following consumption of eggs treated with an illness-inducing toxin. Previous studies suggest that this technique is effective but many have been subject to several methodological flaws that limit their applicability. Here I employ an improved experimental design that uses both spatial and temporal controls and incorporates quantification of predator identity and abundance. By so doing the resultant effects can be attributed to CFA treatment with higher certainty. In the Berg River Estuary, South Africa, nest losses of the Kittlitz's Plover (*Charadrius pecuarius*) are high due to Pied Crow (*Corvus albus*) nest predation. I used this common plover as a model species to test whether CFA can be used as a conservation management tool to reduce nest predation. I used a field experiment to assess whether provisioning quail eggs treated with carbachol, an illness-inducing chemical, resulted in reduced nest predation. To assess the effects of treatment, nest survival data for both artificial plover nests containing quail eggs and natural Kittlitz's plover nests, as well as predator abundance were compared across three experimental phases (pre-treatment, treatment and post-treatment) and according to treatment type (carbachol versus water). Treatment with carbachol was associated with decreased predation rates on artificial nests. Nests survived longer after carbachol-treatment than before, and after carbachol-treatment compared to after water-treatment. Due to a low sample size of natural nests post-treatment, it was not possible to evaluate any effects of treatment on natural nest survival. However, spatial patterns of artificial and natural nest survival were qualitatively similar. Pied Crow were identified as the key predator of both nest types. Crows appeared to partially abandon sites following treatment, as crow sighting rates were lower after carbachol treatments. This study was the first to use a modified CFA technique

and the first study in Africa to test CFA as a tool to reduce nest predation. Results demonstrated that CFA can be induced in Pied Crows causing them to avoid carbachol-treated sites which results in increased artificial nest survival. The study also re-iterates the need to investigate the ecological effects of increasing Pied Crow numbers in certain regions of South Africa as excessive nest predation could be detrimental to bird species such as the near-threatened Chestnut-banded Plover (*Charadrius pallidus*). Further refinement of the technique is recommended to enhance the effect and to increase the likelihood that natural nests are also protected. This study has shown that CFA could be a valuable tool for use in conservation management.



## **INTRODUCTION**

Nest predation has long been recognized as one of the principle constraints on bird breeding success accounting for the majority of all nest failures (Lack 1954; Ricklefs 1969; Martin 1987, 1993; Zanette 2002; Catry & Granadeiro 2006; Ekanayake et al. 2015). Extensive research shows that the negative effects of nest predation can be exacerbated by a variety of factors including human-induced habitat fragmentation and degradation (Martin 1987; Robinson et al. 1995; Hartley & Hunter Jr 1998; Manolis et al. 2002; Evans 2004; Macdonald & Bolton 2008), and the introduction of novel predator species (Savidge 1987; Blackburn et al. 2004; Starling-Windhof et al. 2011; Latorre et al. 2013). The resultant increased predation pressure has led to the decline of numerous bird species worldwide (O'Connor 1991; Bogliani & Bellinato 1998; Macdonald & Bolton 2008). Identifying management strategies to reduce nest predation is consequently a priority for biodiversity conservation (Wallander et al. 2006). In many cases, using means of predator control such as lethal control, (Parr 1993; Conover & Lyons 2003), translocation (Millus et al. 2007) and the use of exclosures (Rimmer & Deblinger 1990; Melvin et al. 1992; Isaksson et al. 2007; Maslo & Lockwood 2009) can be difficult to implement, ineffective, unethical, time-consuming and expensive (Conover 1990; Côté et al. 1997; Bolton et al. 2007; Donehower et al. 2007; Madden et al. 2015). An alternative method, which is potentially more effective and ethically acceptable, is the use of Conditioned Food Aversion (CFA) aimed at eliciting prey avoidance behavior in predators through exposure to similar food items that are distasteful or cause illness (Rogers Jr 1974; Nicolaus et al. 1983; Gustavson & Gustavson 1985; Nicolaus 1987; Dimmick & Nicolaus 1990; Cox et al. 2004; Gabriel & Golightly 2014).

### ***Nest predation and species declines***

On average 80% of all avian nest losses are due to nest predation (Martin 1993b; Chalfoun et al. 2002). This has a major influence on population trends of many species around the world (Massei et al. 2002). In particular, ground-nesting species have shown some of the most consistent long-term population declines as a result of nest predation and other exacerbating factors associated with habitat change (Martin 1993a; Macdonald & Bolton 2008; Amar et al.

2011). Research shows that high nest predation pressure can lead to severe population declines and limit population recovery (Martin 1998; Craik 1997; Ibáñez-Álamo et al. 2015). In the most extreme cases, populations and species have been driven to extinction as a result of excessive nest predation pressure (Savidge 1987; Massei et al. 2002; Towns et al. 2006; Starling-Windhof et al. 2011). This has commonly been observed on islands, where some introduced predators have become invasive due to release from natural enemies and because they can prey easily on naïve bird species which have not evolved traits to reduce predation risk (Starling-Windhof et al. 2011; Latorre et al. 2013). Mammals including mice, rats and cats (Blackburn et al. 2004), and reptiles such as snakes (Savidge 1987), all present a major threat to avifauna of islands where they have been introduced. In New Zealand, at least 42 native bird species have been driven to extinction, most of them due to excessive predation by invasive mammals (Towns et al. 2006; Starling-Windhof et al. 2011). On the island of Guam, 9 out of 12 species of forest birds are now extinct in the wild as a direct consequence of predation of eggs, chicks and adults by the brown tree snake (*Boiga irregularis*) introduced from New Guinea in the 1950s (Savidge 1987; Engeman et al. 2000).

Native predators can also pose a threat to vulnerable prey species (Fletcher et al. 2010) especially when they are 'overabundant' (Côté et al. 1997). In combination with human induced rapid environmental change (HIREC) (Hethcoat & Chalfoun 2015), increased predation from generalist predators in particular has been recognized as a major cause of population decline in many bird species (Bodey et al. 2011). Of the generalist predators, corvids represent a large and successful group of egg predators (Catry & Granadeiro 2006; Madden et al. 2015) and can be detrimental to species of conservation concern (Avery & Decker 1994). Furthermore, global populations of these highly adaptable animals are growing and expanding (Fletcher et al. 2010; Peery & Henry 2010; Ekanayake et al. 2015) most likely because corvids often thrive near areas of human settlement (Marzluff & Neatherlin 2006; Cunningham et al. 2015), in heavily modified (Goodrich & Buskirk 1995; Stien et al. 2010) or fragmented landscapes (Andrén 1992). Additionally, nest predation by corvids is often heightened because they can exploit alternative food sources during the non-breeding season (Ekanayake et al. 2015). Although there is mixed evidence as to effects that corvids have on biodiversity (Madden et al. 2015), the negative

impacts of corvids can be severe in some cases, especially on threatened and endangered species (Catry & Granadeiro 2006; Peterson 2013; Madden et al. 2015) and when corvid abundance is unusually high (Madden et al. 2015). Thus there is concern that the current rise in corvid numbers may accelerate the decline of already small populations of vulnerable species, in some cases even to the point of extinction (Parr 1993; Catry & Granadeiro 2006; Ekanayake et al. 2015).

### ***Predator control: lethal and non-lethal***

Predator control has an important role to play in the conservation of threatened species of birds (Côté et al. 1997; Fletcher et al. 2010). A variety of both lethal and non-lethal approaches are available (Goodrich & Buskirk 1995; Conover & Lyons 2003; Isaksson et al. 2007). However, not all approaches are created equal. Successful outcomes depend on a variety of factors including the mode of control, means of implementation, biology of both predator and prey species as well as the environmental and social context in which control is implemented (Stien et al. 2010). Consequently, conservation efforts to control predators have shown mixed results (Côté et al. 1997).

Lethal approaches are the most commonly used means of predator control (Goodrich & Buskirk 1995). The negative effects of invasive predators on native birds have precipitated major eradication efforts on several islands around the world (Engeman et al. 2000; Medina et al. 2011; Russell et al. 2015). Some attempts to eradicate invasive predators on islands have successfully prevented extinctions (Butchart et al. 2006) and restored ecosystem functionality (Russell et al. 2015), mainly because re-colonisation can be prevented more easily in closed island systems than in mainland areas. Systematic trapping and poisoning of invasive mammalian predators significantly increased nest survival of native birds and lead to the recovery of several populations in New Zealand (Starling-Windhof et al. 2011). Trapping has also helped to control the population of brown tree snakes in Guam (Engeman et al. 2000). However, results are mixed and sometimes widespread eradication efforts are not feasible, either economically or environmentally (Latorre et al. 2013). Nevertheless, when implemented

correctly, lethal control can be effective and is sometimes imperative to protect threatened native species from non-native species, especially on islands (Russell et al. 2015).

Lethal control of abundant native predators on the mainland is more complex (Côté et al. 1997). Not only is it often met with resistance based on ethical grounds, but it is often difficult to implement effectively on a large scale (Côté et al. 1997; Catry & Granadeiro 2006) and does not always produce the desired results (Goodrich & Buskirk 1995). Several interacting factors limit the efficacy of lethal control, such as the potential to trigger meso-predator release (decrease in abundance of one predator leads to increased abundance of another) or allow for compensatory predation (reduced predation by one species allows for increased predation by another) (Madden et al. 2015). There is also the possibility of rapid population recovery or even population increases amongst predators due to density-dependent factors affecting reproduction, dispersal or mortality (Goodrich & Buskirk 1995; Conover & Lyons 2003). Strategies such as shooting are logistically difficult and often fail to eliminate more elusive predators (Goodrich & Buskirk 1995) or individuals that become wary of humans (Donehower et al. 2007). Furthermore, the high costs involved, negative effects of poisoning or trapping on non-target species and major ethical issues all present further obstacles to the use of lethal predator control (Goodrich & Buskirk 1995).

It must be noted that in certain cases lethal predator control is necessary and can be used successfully to protect threatened species from native predators (Goodrich & Buskirk 1995). Several efforts to remove crows have led to improved breeding success of ground-nesting birds (Fletcher et al. 2010). In a review on corvid impacts, Madden et al. (2015) draw attention to the fact that the removal of corvids together with other predators is more likely to improve productivity than removing corvids alone. Stien et al. (2010) showed that Common Eider (*Somateria mollissima*) hatching success improved by 19% after the removal of Hooded Crows (*Corvus cornix*) at one colony, however, crow removal at another colony nearby had no positive effect on breeding success. This was because compensatory predation by other predators in the area (mink, otter and white-tailed eagle) that may have benefited from the increased food availability, limited the effects of crow removal. Similarly, compensatory predation by Common Ravens (*Corvus corax*) masked any increases in hatching success of Northern Lapwings (*Vanellus*

*vanellus*) as a consequence of Hooded Crow and Ferret (*Mustela furo*) removal (Bodey et al. 2011). Furthermore, the removal of Carrion Crows (*Corvus corone*) and Common Gulls (*Larus canus*) did not improve the hatching success of a declining population of Golden Plovers (*Pluvialis apricaria*) probably due to compensatory predation by foxes (*Vulpes vulpes*) (Parr 1993). In most cases, it is not possible to adequately reduce the abundance of all potential predators and therefore alternative methods for the protection of prey species must be sought.

Non-lethal means of predator control provide alternative and perhaps better solutions to address excessive predation pressure on ground-nesting birds (Goodrich & Buskirk 1995). These include measures such as exposure to unrewarding prey cues (Price & Banks 2012), use of spray repellents (Conover & Lyons 2003), startle devices (Cox et al. 2004), effigies (Peterson 2013), predator exclosures and aversive conditioning (Goodrich & Buskirk 1995). Here again, some methods are more effective than others. Price & Banks (2012) show that the placement of unrewarding cues could be useful to protect prey as rats began to ignore unrewarding odor cues that simulated birds' nests, suggesting that by association they may ignore real nests too. Spraying eggs with taste repellents may not be a viable option because even if cryptic nests can be found, the repellent must be consumed to be effective and predators usually eat the contents of the eggs, not the shells (Conover 1984). Startle devices may not work if predators become accustomed to them (Conover 1979) and in some cases the placement of effigies may even be counterproductive. Corvids were undeterred by effigies hung near Snowy Plover (*Charadrius nivosus*) nests and instead the effigies attracted humans and their dogs putting nests of this threatened species at higher risk (Peterson 2013). Predator exclosures (wire cages or electric fences) can effectively protect nests from large mammalian and some avian predators (Rimmer & Deblinger 1990; Melvin et al. 1992). However they have been known to actually decrease hatching success by attracting predators to nests (Niehaus et al. 2004), hindering the escape of an incubating adult (Isaksson et al. 2007; Barber et al. 2010) or inducing nest abandonment (Isaksson et al. 2007; Maslo & Lockwood 2009; Barber et al. 2010).

Another non-lethal strategy for reducing nest predation is the use of food-avoidance conditioning (Avery & Decker 1994). This approach takes advantage of the learning capacity of animals to modify predator behavior through the provision of baits that mimic natural cues

such as nests or prey and cause temporary illness (Nicolaus et al. 1983; Gustavson & Gustavson 1985; Nicolaus 1987; Dimmick & Nicolaus 1990; Cox et al. 2004; Šálek & Zámečník 2014; Tapilatu et al. 2015) or provide no reward (Price & Banks 2012). Conditioned aversion trials to this effect in North America, Australia and Europe have successfully reduced nest predation rates by a variety of both mammalian (Maguire et al. 2009; Massei et al. 2002; Price & Banks 2012; Martin 2007) and avian predators (Avery et al. 1995; Gabriel & Golightly 2011; Dimmick and Nicolaus 1990; Bogliani and Bellinato 1998). These and other studies indicate that this technique could be a more effective and ethically acceptable alternative of predator control for use in conservation (Avery & Decker 1994; Gill et al. 2000; Cox et al. 2004; Maguire et al. 2009).

### ***Conditioned Food Aversion (CFA)***

Conditioned Food Aversion (CFA) is based on a natural phenomenon whereby animals can learn to avoid food items that make them sick (Nicolaus et al. 1983). This is the most widespread form of learning in animals (Gustavson 1977) and probably evolved in order to reduce the risks of poisoning (Garcia & Hankins 1977). When applied to conservation, predators can be deliberately deterred from vulnerable prey if baited with similar food items containing an illness-inducing compound (Avery et al. 1995; Catry & Granadeiro 2006). The technique is often referred to as Conditioned Taste Aversion (CTA) although this can be misleading as tasteless aversion agents are often used for treating bait. In this study, I used carbachol, a chemical believed to be colourless, odourless and tasteless to predators (Gabriel & Golightly 2014) and therefore preferred to use the term Conditioned Food Aversion (CFA) (Gustavson & Gustavson 1985; Conover 1990).

In order to reduce egg predation in particular, predators can be deliberately induced to avoid certain eggs when provisioned with similar eggs treated with a substance that makes them ill (Nicolaus et al. 1983; Dimmick & Nicolaus 1990; Martin 2007). Theory suggests that upon eating an egg that makes them sick, they will develop an aversion to treated eggs, an effect which will spillover to untreated eggs and therefore all eggs of that type will subsequently be avoided (Conover 1990). Ideally, the illness must develop quickly, and be severe enough for the

individual to make the association between the egg(s) and sickness after just one or two sickness episodes (Nicolaus et al. 1983; Gill et al. 2000). Additionally, the sickness must be mild enough to avoid any long-term negative effects in both target and non-target species (Conover 1990; Gill et al. 2000). The time taken to develop such an aversion as well as the strength and latency of the effect, are dependent on the chemical, dosage, and food item used (Gill et al. 2000; Massei & Cowan 2002) as well the length of the exposure time (Catry & Granadeiro 2006; Maguire et al. 2009). Laboratory tests with captive individuals are typically used to determine what these should be (Avery & Decker 1994; Cox et al. 2004).

### ***CFA laboratory trials***

There are numerous benefits associated with monitoring predator responses to an emetic, an illness-inducing substance, in a controlled environment such as a laboratory. Hence laboratory trials have been used to compare different emetics (Gill et al. 2000; Massei & Cowan 2002), determine safe and effective dosages (Cox et al. 2004; Gabriel & Golightly 2014), identify the minimum amount of time needed for aversions to be acquired and how long they can be retained (Gabriel & Golightly 2014). Such trials are important to determine whether CFA can be induced in a target predator species whilst remaining within the confines of ethical acceptability. Laboratory tests have confirmed that many nest predators can be taught to avoid untreated eggs using various CFA techniques (Rogers Jr 1974; Nicolaus 1987; Massei et al. 2002; Cox et al. 2004; Gabriel & Golightly 2014). Various effects point to the successful development of aversion, including a reduction in the total number of eggs eaten (Nicolaus et al. 1983), the actual amount eaten per egg, or the time to predation, known as attack latency (Cox et al. 2004).

Although useful, laboratory studies alone cannot answer the question of whether or not CFA is an effective tool for use in practical conservation management. This is because the responses of captive individuals are not necessarily representative of what might happen in the wild under unpredictable ecological conditions where numerous factors could influence the overall outcome of CFA treatment (Gustavson & Gustavson 1985; Nicolaus 1987). Such variables

include predator densities, movement patterns, foraging behavior, social structure, interspecific competition for food resources, and availability of alternative or more preferable food (Rogers Jr 1974; Gustavson & Gustavson 1985). These in combination with the density and spatial arrangement of treated food items are all factors that could influence the amount of treated food eaten and therefore the actual dosage consumed by predators (Rogers Jr 1974; Gustavson & Gustavson 1985). Hence there is also a need for rigorous testing of CFA techniques under natural conditions. CFA field trials are used where this is concerned, which in some cases incorporate findings from prior testing in a laboratory in their design (Nicolaus 1987; Cox et al. 2004; Gabriel & Golightly 2014).

### ***CFA field trials***

Findings from numerous field trials provide evidence that CFA can also be induced in free-ranging predators. Several different mammal species have been targeted in the past, including foxes (Maguire et al. 2009), rats (Massei et al. 2002; Price & Banks 2012), and raccoons (Martin 2007). In addition, bird species from the family Corvidae have been commonly targeted, for example ravens (Avery et al. 1995), Steller's Jays (Gabriel & Golightly 2011), American Crows (Nicolaus et al. 1983; Dimmick & Nicolaus 1990) and Hooded Crows (Bogliani and Bellinato 1998). CFA in wild predators such as these is achieved through the provision of treated bait in areas of natural habitat where the predators of concern are known to occur, often in high densities (Nicolaus 1987).

Although experimental designs differ according to context, in general they include separate treatment and control sites (Nicolaus 1987; Dimmick & Nicolaus 1990), periods (Conover 1990; Avery et al. 1995), bait types (Nicolaus 1987; Bogliani & Bellinato 1998; Gabriel & Golightly 2014) and various combinations of these (Dimmick & Nicolaus 1990; Avery et al. 1995). Treatment, otherwise known as conditioning (Bogliani & Bellinato 1998; Gabriel & Golightly 2014), includes the provision of bait treated with an aversive agent, whereas for controls, baits are untreated (Nicolaus 1987). Once distributed in a site, baits are checked for predation regularly, every few hours (Nicolaus 1987; Nicolaus et al. 1989; Avery et al. 1995), daily



(Conover 1990; Bogliani & Bellinato 1998), every few days (Maguire et al. 2009) or once after a certain number of days (Gabriel & Golightly 2014). Comparisons are then made between treatment and controls to determine the efficacy of the treatment. If treatment predation rates are significantly lower than those for controls, CFA is thought to have been successfully induced (Nicolaus 1987).

Where nest predation is concerned, typically CFA experimental designs involve the creation of artificial nests that simulate natural nests. Similarity between the treated bait and the food (prey) in need of protection is important such that predators might mistake the latter for the former (Conover & Lyons 2003). In line with this, quail eggs are commonly used when attempting to use CFA to protect ground-nesting species because they are similar in size, shape, colour and patterning to the eggs of many ground-nesting birds (Avery et al. 1995; Maguire et al. 2009; Šálek & Zámečník 2014). Additionally nests are created in or near known breeding habitat of the species at risk, such as in a breeding colony (Avery et al. 1995; Bogliani & Bellinato 1998), along beaches (Maguire et al. 2009) or in forests (marbled murrelet, Gabriel & Golightly 2014) and in many cases trials are conducted to overlap with breeding season when the risk of predation is most apparent (Maguire et al. 2009).

Past CFA field trials using artificial eggs as bait suggest that the technique can be used to reduce egg predation in several corvid species. Dimmick & Nicolaus (1990) showed that predation of both treated and untreated eggs by wild American Crows (*Corvus brachyrhynchos*) was significantly suppressed after they consumed eggs treated with the cholinesterase inhibitor Landrin. Later Bogliani & Bellinato (1998) used carbachol treated eggs in a CFA trial and found that predation of treated eggs was significantly lower than untreated eggs. Hooded Crows (*Corvus corone cornix*) were identified as the main egg predator in the area where their trial was conducted. More recently Gabriel & Golightly (2014) successfully conditioned wild Steller's Jays (*Cyanocitta stelleri*) using carbachol treated eggs that mimicked Marbled Murrelet (*Brachyramphus marmoratus*) eggs, such that predation of treated eggs was as much as 37% lower than predation of control eggs. These studies confirm that CFA could be a valuable tool for conservation to reduce high rates of nest predation by corvids. However several methodological flaws limit the robustness of these studies. **These include the use of inadequate**

controls, the potential to actually increase predation where CFA is not effectively induced, failure to adequately quantify predator-specific variables and lack of attempts to evaluate the effects of treatment on natural nests.

### ***Methodological flaws of previous CFA field trials***

One of the most important methodological flaws made in CFA trials to date is the use of inadequate controls, both qualitatively and quantitatively. This casts doubt on the reliability of the conclusions drawn from these studies. For example, Dimmick & Nicolaus (1990) attempted to use a control by comparing predation by American Crows at geographically separate treatment and control sites. Although such an approach ensures that predators in different sites are exposed to different treatment types, predation rates are still prone to biases associated with site-level differences in predator numbers for which no control is used. One way of minimizing the effect of site-level differences is to place both treated and control eggs in one site. However, one study that used this strategy by placing eggs of different colours that suffered equal predation probability by Hooded Crows, used only a single site (heron colony) (Bogliani & Bellinato 1998), highlighting the issue of insufficient replication. Poor replication limits the generality of the results, as treatment effects may be limited to an unrepresentative subset of the predator population. Even in rare cases where differences between sites are inconsequential, often only a few sites are incorporated. Maguire et al. (2009) used four closely spaced nest arrays (1 km apart) per treatment type when attempting to deter foxes (*Vulpes vulpes*) from predating artificial plover nests. This again highlights the issue of insufficient replication as it is probable that only a few individual predators were affected.

Another issue that few if any field trials have addressed is the possibility that emetic eggs may even increase predation versus control eggs. This could occur where CFA is not induced and provisioning eggs instead boosts the available foraging returns from nest predation, thereby increasing such behaviour. CFA might fail where the emetic is ineffective, the dosage is too low, or the frequency of encounters by individual predators is insufficient. Additionally despite the negative consequences of consuming emetics, the efficacy of CFA is dependent on the ratio of

emetic eggs to normal eggs, in accordance with the theory of Bayesian mimicry (Nicolaus et al. 1983; Bogliani & Bellinato 1998). CFA is therefore unlikely to develop if the ratio of emetic to normal eggs is too low and predators benefit from continuing to eat eggs on average (Bogliani & Bellinato 1998). Furthermore, where payoffs from alternative food sources are low, and emetic eggs only mildly costly, it may even pay for predators to consume emetic eggs regardless. Therefore to avoid unintended boosting of predation it is essential to effectively induce CFA through deployment of an adequate number of eggs (Dimmick & Nicolaus 1990) containing an optimal dosage of chemical that induces illness severe enough to induce avoidance (Nicolaus et al. 1983; Gill et al. 2000).

If implemented properly, CFA can be induced in all predator species in the system, eliminating the problems associated with compensatory predation (see above) and increasing the chances of successfully reducing predation. However, one cannot ensure this nor assess whether it has been achieved by relying only on measures of nest survival as an indicator of the effect of treatment. Many previous CFA trials have done this, neglecting to adequately quantify predator-specific variables such as predator identity, temporal and spatial differences in abundance or behavioral modifications as a result of treatment (Conover 1990; Bogliani & Bellinato 1998; Conover & Lyons 2003; Price & Banks 2012; Gabriel & Golightly 2014). These are essential to our understanding of how to develop appropriate and effective CFA protocols regarding optimal dosages (Nicolaus et al. 1983; Gill et al. 2000), quantities of bait required and suitable bait arrangements in the field (Dimmick & Nicolaus 1990). They can also be used to determine whether any changes in predation are due to the treatment itself or other factors that affect variability in predation pressure, for example seasonal movements or temporal changes in abundance through the breeding season (Gustavson & Gustavson 1985) or simply site level differences in predator identity and densities (Conover & Lyons 2003). Some have attempted to identify potential predators from visible animal tracks (Nicolaus et al. 1989; Conover 1990; Avery et al. 1995), remnants of hair or based on the type of bait damage (Conover 1990; Conover & Lyons 2003; Gabriel & Golightly 2014). Few studies go further to consider predator abundance and movement patterns (Nicolaus et al. 1983, 1989; Dimmick & Nicolaus 1990). Following free-ranging predators to observe individual responses to an emetic

is a practical challenge. However, other measures such as predator visitation rates could be used as a proxy for behavior. Chalfoun et al. (2002) point out that conservation efforts must be tailored to suit the predator accountable for high rates of nest failure, and thus there is a need for CFA field trials to pay closer attention to the predators themselves.

Perhaps the most notable shortcoming of CFA field trials to date is that they have neglected to assess the effects of provisioning emetic eggs on natural nests. Those that provide some indication that the nearby placement of treated eggs confers some protection to natural nests once again lack comparison with controls (Conover 1990; Avery et al. 1995). Without these controls, it is impossible to know whether variation in nest predation is attributable to the presence of an emetic or some other factor such as natural variation in predator numbers. This highlights an important gap in our understanding of the utility of CFA as a tool for conservation, and presents an opportunity for further refinement of the technique. If CFA is to become a tool for use in practical conservation, its ability to afford protection to natural nests must be demonstrated.

In summary, many previous attempts to test the use of CFA in natural environments to reduce nest predation have failed to do so due to several methodological flaws. This provided an opportunity to address past failures and improve the technique. In this study, I attempted to improve the methodology by addressing i) the inadequacies of site controls, ii) the insufficient quantification of predator abundance, and iii) the lack of assessment of protection afforded to natural nests. To do this I used a design that incorporated geographically separate treatment and control sites, using both a pre-treatment phase and post-treatment phase to control for site-specific differences and effects e.g. differences in background predation rates. I quantified predator abundance throughout the experimental period to test mechanistic explanations if CFA was induced. I also attempted to monitor natural nests to determine whether predation on artificial nests is qualitatively similar and whether natural nests are afforded any protection at all.

### ***Pied Crows, plovers and CFA***

Although the Pied Crow (*Corvus albus*) is the most common corvid in Africa, little is known about the ecological importance of this species relative to other corvids elsewhere in the world (Madden 2013). Recently, researchers and conservation organisations such as Birdlife South Africa have begun drawing attention to this knowledge gap, emphasizing the need for more studies on the ecological impacts of Pied Crows (Fincham & Lambrechts 2014; Cunningham et al. 2015; Fincham et al. 2015; Madden et al. 2015). As a generalist predator (Cunningham et al. 2015) Pied Crows impose heavy predation pressure on a variety of prey species and there is some anecdotal evidence to suggest that they could pose a major threat to threatened species such as the range-restricted angulate tortoise (*Chersina angulate*) (Fincham & Lambrechts 2014). To make matters worse, Pied Crow numbers are thought to be increasing in South Africa as a result of global warming and other anthropogenic factors including the availability of nest sites on electrical infrastructure, increased food availability in urban areas and from road kill (Dean & Milton 2003; Cunningham et al. 2015). In combination, these factors have also allowed the Pied Crow to expand its distribution range and persist in previously unsuitable habitats (Cunningham et al. 2015). Given that the Pied Crow is an efficient and competitive avian predator this shift in distribution has raised concerns about what ecological consequences could result (Cunningham et al. 2015).

In 2013, researchers working in the Berg River Estuary in the south-western part of the country where Pied Crow numbers are thought to be increasing, identified the Pied Crow as the principle predator at nests of three species of wader (Charadriiformes): Kittlitz's Plovers, Chestnut-banded Plovers (*Charadrius pallidus*), and Blacksmith Lapwings (*Vanellus armatus*) (Troscianko et al. in prep). Of the 27 Kittlitz's Plover nests whose fate was known, only 3 survived to hatch, 18 were depredated and the rest failed due to unknown causes, high water levels or abandonment. Ground-nesting birds including waders may be especially vulnerable to predation given that their nests are often less concealed, more accessible and exposed to many types of predators (Martin 1993a; Ekanayake et al. 2015) and studies show that predation can severely limit breeding success (Martin 1993a; Fletcher et al. 2010). Given that wader nest failure is thought to be an important demographic parameter (Macdonald & Bolton 2008), high

rates of nest predation could be detrimental to populations of wader species such as the 'near threatened' Chestnut-banded Plover (Birdlife International 2016). Both the Chestnut-banded Plover and the Kittlitz's Plover are highly localized breeders (Hockey et al. 2005) suggesting that CFA techniques could be used to reduce predation in key breeding areas that are easy to identify and manage. As such the Berg River Estuary provided an ideal location to test the use of CFA as a potential conservation tool to reduce plover nest predation using the common Kittlitz's Plover as a model species and targeting mainly the Pied Crow as the major predator.

The Pied Crow fits the criteria of a predator species suitable for CFA (Nicolaus & Nellis 1987). Pied Crows show little variation in body size (550g, Hockey et al. 2005) suggesting that individuals have similar meal sizes allowing for the use of a general dosage of an emetic based on the average body size of the species. They are generalist predators (Cunningham et al. 2015) feeding on a wide range of food items in addition to eggs including chicks, insects (beetles, locusts, termites), fish, small mammals, small reptiles (snakes, tortoises, lizards), carrion, plant material (seeds, crops, roots, nectar), ectoparasites domestic scraps (Hockey et al. 2005). Their ability to utilize several different food sources probably exacerbates their impacts on prey via nest predation (Ekanayake et al. 2015) and also means the removal of plover eggs from their diet is unlikely to have any seriously detrimental effects on individuals. During the breeding season (August to November) they are territorial, remaining close to nest sites which they actively defend (Winterbottom 1975). This means that CFA can be induced in specific individuals likely to be responsible for most of the nest predation at a given site and that the potential for compensatory predation by other unexposed individuals is minimized, thereby enhancing the CFA effect (Avery et al. 1995). Crows also have well-developed cognitive abilities such that they can learn and learn rapidly (Winterbottom 1975; Marzluff & Neatherlin 2006). Lastly, CFA aversion has already been successfully induced in several other crow species (Dimmick & Nicolaus 1990; Avery & Decker 1994; Bogliani & Bellinato 1998) suggesting that the method could be successfully applied to reduce predation rates by Pied Crow.

## ***Research aims***

The study aimed to assess whether provisioning eggs laced with an emetic (illness-inducing chemical) to induce Conditioned Food Aversion (CFA) in predators, primarily Pied Crow, could be used as a conservation management tool to reduce predation of plover nests. To do this the following research questions were addressed:

1. Does provisioning eggs treated with carbachol (an emetic) increase survival of artificial plover nests in a natural environment?
2. Is this protection also afforded to natural Kittlitz's Plover eggs?
3. Is CFA induced in wild Pied Crows that consume treated eggs?

To answer these questions, I used an experimental design that was similar to previous studies, but modified to address some of the inadequacies of previous trials. A field experiment was conducted to determine whether provisioning emetic (carbachol treated) or control (water filled) eggs in a natural environment reduced predation of artificial nests in the period following treatment versus the period preceding treatment. I also assessed natural nest survival to test whether artificial nests are suitably comparable, and in an attempt to assess whether natural nests receive any protection from the provisioning of emetic eggs. Nest cameras were placed at artificial and natural nests to identify predators and assess if the Pied Crow was the principal predator. In the absence of observations on changes in the behaviour of individual predators in response to consumption of emetic eggs, predator responses to treatment were evaluated indirectly based on changes in predator abundance at the sites.

## METHODS

### *Study area*

Research was undertaken from the 25<sup>th</sup> September to the 27<sup>th</sup> November 2015 at the Berg River Estuary (32°51'S, 18°16'E) located 140 km north of Cape Town near the town of Velddrif in the Western Cape Province of South Africa (Fig. 1). The floodplain consists of eight wetland types, namely ephemeral pans, commercial saltpans, reed-marsh, sedge-marsh, salt-marsh, halophytic floodplain, xeric floodplain and intertidal mudflats (Barnes 1998). Approximately 250 bird species have been recorded in the area including 127 water-birds (Barnes 1998). During summer, the estuary supports over 12000 non-passerine water-birds whereas in winter there are about 6000 individuals (Barnes 1998). This highly productive coastal wetland is recognized as one of 124 Important Bird Areas (IBAs) in South Africa (Birdlife South Africa, 2015) owing to the fact that it provides key habitat for an abundance of both resident and migratory water-birds (Velasquez et al. 1991; Velasquez 1992). These include common species such as the Kittlitz's Plover (*Charadrius pecuarius*) as well as species at risk such as the regionally vulnerable Painted Snipe (*Rostratula benghalensis*) and globally near-threatened Chestnut-banded Plover (*Charadrius pallidus*) which breed there (Barnes 1998).

The Berg River Estuary provided an ideal location for a field trial of CFA due to: 1) a large population of ground-nesting resident waders such as the Kittlitz's Plover as well as the less abundant, more threatened Chestnut-banded Plover, whose nests and eggs can be reasonably well mimicked according to the human eye using locally available quail eggs, 2) a high abundance of potential egg predators such as the Kelp Gull (*Larus dominicanus*) and the Pied Crow (*Corvus albus*), the latter of which has shown increased reporting rates in the past two decades (Cunningham et al. 2015), and 3) existing evidence from previous research in the area suggesting high rates of Kittlitz's Plover nest predation predominately by the Pied Crow (Troscianko et al. in prep).

Six sites were available (Fig. 1) for use in the CFA trial which was conducted from the 2nd October to 26th November 2015, to overlap with the breeding season of the Kittlitz's Plover (Johnsgard 1981; Hockey et al. 2005). Sites were chosen in suitable Kittlitz's Plover breeding





Figure 1. Map showing the location of six sites (A - F) used for the Conditioned Food Aversion (CFA) trial in the Berg River Estuary, South Africa.

habitat and were geographically separated by at least 2 km. A maximum of six sites were available and fitted this criteria. Furthermore, six sites was also the maximum number of sites that could be monitored effectively by one observer. Four of the sites (A, B, C and E) were located within or adjacent to commercial salt pans and the remaining two sites (D and F) were on salt-marshes on private farmland. Permission to use the land for this experiment was granted by CapeNature (permit number: 0056-AAA041-00126), as well as the relevant landowners. Ethical clearance for the provisioning of carbachol-treated eggs at the identified dosage (see below) as well as to monitor Kittlitz’s Plover nests (see below for methodological

protocol) was obtained from the UCT Science Animal Ethics Committee (Number 2015/V13/AF) and CapeNature (permit number: 0056-AAA041-00126).

### ***Study species and population***

The Kittlitz's Plover has a wide distribution, extending from the Nile River Delta in north Africa, southwards throughout sub-Saharan Africa into southern Africa as well as in Madagascar (Tree 1997). It is a locally common species across southern Africa, with the exception of drier areas in central Botswana, Namibia and the Northern Cape Province of South Africa (Tree 1997; Hockey et al. 2005). Its preferred habitat consists of open dry mudflats with short grass near water including salt-marshes, natural pans, commercial salt pans, estuaries and adjacent floodplains (Tree 1997; Hockey et al. 2005). It is the most abundant resident wader in the Berg River Estuary (Barnes 1998) with the highest number of birds (3676 birds) recorded in January 1976 (Summers et al. 1977). Its diet consists of seeds, a variety of small invertebrates including insects and their larvae, crustaceans, molluscs and polychaete worms (Johnsgard 1981; Kalejta 1993; Hockey et al. 2005).

In the winter rainfall region where the study site is located, breeding season for the Kittlitz's Plover begins after the rains have stopped in July/August and extends to March/April, peaking from October to January (Johnsgard 1981; Hockey et al. 2005). Breeding occurs most often on open shorelines near to water (Hockey et al. 2005), but on commercial salt pans birds often make their nests on raised dykes between concentration and crystallization pans (A. Ferguson pers. obs.). This ground-nesting species lays its eggs in shallow nest scrapes approximately 3 cm deep, 11 cm in diameter and 8 to 40 m apart (Johnsgard 1981; Hockey et al. 2005). Scrapes are dug in coarse sand, dried mud or on a raised mound, and are often lined with sand pebbles, small pieces of vegetation, mud, grass, broken shells or animal dung (Hockey et al. 2005, Fig. 2a – c). Between 1 and 3 eggs (usually 2 per clutch) are laid at 1 to 2 day intervals, each measuring about 31.2 x 22.1 mm with an average mass of 8.7 g. They are oval shaped and elongate, varying in colour from dull white to cream with fine black lines, 'scrawls' and dark spots (Johnsgard 1981; Hockey et al. 2005, Appendix A). Both sexes incubate (female during the day,

male at night) and eggs are incubated for 22 to 28 days after the last egg has been laid (Hockey et al. 2005).

When disturbed, incubating adults are known to kick sand and other loose material over the eggs (Hockey et al. 2005), although the degree of egg covering is highly variable (0 to 100%) and seldom occurs during normal nest relief (Johnsgard 1981). A variety of predator distraction displays are often performed, including 'broken-wing' behavior, 'rodent-run' and 'false brooding' (Johnsgard 1981; Hockey et al. 2005). After hatching, the egg shell remains are removed by one of the parents. Chicks leave the nest within 24 hours to be brooded by both parents for up to 6 weeks even though they fledge (become independent foragers) after approximately 4 weeks (Johnsgard 1981; Hockey et al. 2005).

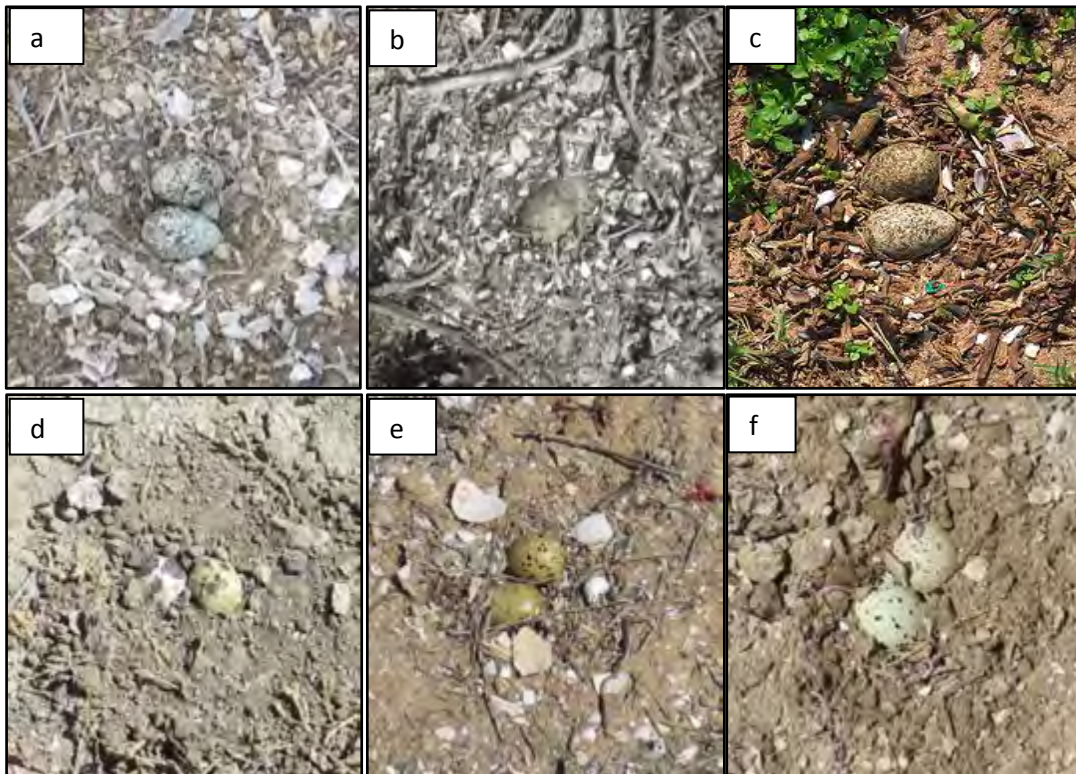


Figure 2: Comparison of exposed natural Kittlitz's Plover (*Charadrius pecuarius*) nests (top a - c) and artificial plover nests containing Japanese quail (*Coturnix japonica*) eggs (bottom d - f), in the Berg River Estuary, South Africa. Photographs by Angela Ferguson except c) by Jolyon Troschianko, BBSRC, Exeter University.

### ***CFA experimental design***

To test whether provisioning eggs treated with an emetic can reduce nest predation, I presented and monitored predation of artificial nests containing quail eggs similar in appearance to Kittlitz's Plover eggs and nests. The experiment was split into three phases: pre-treatment, treatment and post-treatment, each lasting 14 days in keeping with the time taken to acquire aversion in previous studies (Dimmick & Nicolaus 1990; Cox et al. 2004). During the treatment phase, 'emetic' eggs injected with a carbachol and egg mixture were provisioned at three of the sites whereas 'control' eggs injected with water were presented at the other three sites (Fig. 3). Carbachol was used to quickly induce a temporary illness in any predator within a few minutes (Nicolaus et al. 1989) following the ingestion of an emetic-laced egg with development of symptoms such as diarrhoea, vomiting, salivation and retching (Gabriel and Golightly 2014). Water-filled eggs were presumably a more suitable control than untreated eggs as they were neither rewarding nor a deterrent to predators and were therefore unlikely to influence predator behavior. To minimise any effect of temporal differences in predation rate and maintain a continuous availability of eggs in the environment, the interval between phases was kept to a minimum at all six sites: 5 days between pre-treatment and treatment, and 1 day between treatment and post-treatment. The same phase was run concurrently at all sites with maximum of six days between the onset or completion of a phase at different sites.

Twenty artificial nests each containing two untreated quail eggs were provisioned at all six sites during the pre-treatment and post-treatment phases (Fig. 3). This enabled comparison of nest survival and predation pressure before and after the treatment and to assess the latency of any effect. During the treatment phase, sites were provisioned with 30 artificial nests each containing two quails treated with either carbachol or water as detailed above. Initially, 20 nests were provisioned in the treatment phase, but following rapid early predation of eggs I decided to replace nests at every other check until 10 nests had been replaced in each plot. This prolonged exposure time to treated eggs whilst still ensuring that nest density and therefore detectability were not altered excessively. Replacement nests were created in new locations at least 20 m away from any intact nest in keeping with the experimental protocol (see below) and at least 5 m away from any previously predated nest to minimize the risk that

predation rates for replacement nests may have been higher as a result of a predator returning to a learned nest location (Fenske-Crawford & Niemi 1997).

**Plot design**

The six sites used were located in suitable Kittlitz’s Plover nesting habitat and plots created therein. Plots were 35000 m<sup>2</sup> in size and rectangular although plot dimensions differed slightly at some sites to accommodate site-specific constraints and ensure that there was enough land space to create twenty artificial nests per plot at the chosen density (Plots B, D and F were each 250x140m, Plot A and E were each 200x175m and Plot C was 350x100m).

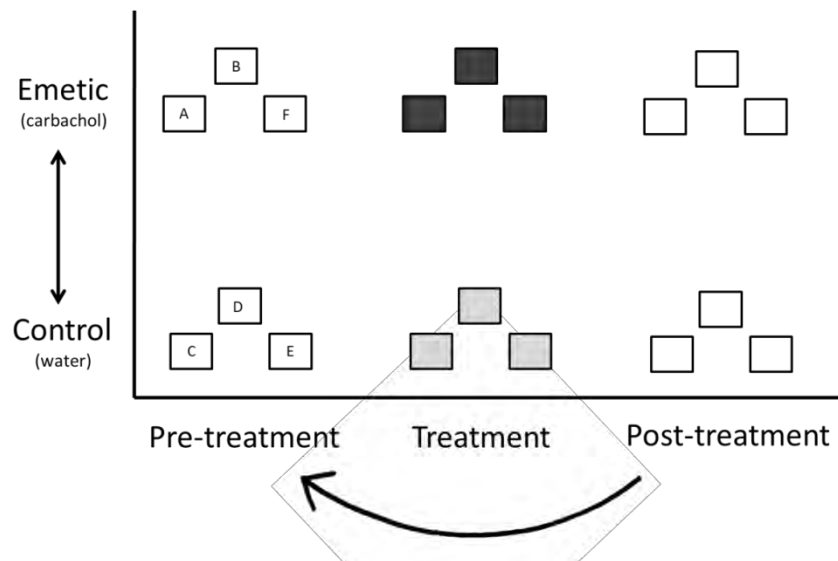


Figure 3: Experimental design of the CFA trial showing the two different treatment types (left) and three treatment phases (below). Arrows indicate comparisons made during data analysis. Squares represent sites, which remained the same throughout the study. Artificial nests containing unmanipulated eggs (empty squares) were provisioned at all sites during pre-treatment and post-treatment. During treatment, artificial nests containing eggs filled with a carbachol and egg mixture (black squares) were provisioned at sites A, B and F, and water-filled eggs were provisioned at control sites C, D and E).

## **Artificial nests**

### **i) Creation**

At the start of each phase, twenty artificial nest scrapes were created within each plot. New nests were created in different locations at the start of each new phase in order to reduce biases associated with predators' ability to learn nest locations (Fenske-Crawford & Niemi 1997; Šálek & Zámečník 2014). Potential nest locations were identified from Google Earth (AfriGIS Pty Ltd, US Department of State Geographer) images prior to set-up and used as a guide. However, due to plot-level constraints, specific nest positions were determined in the field. Nest locations were recorded on a handheld GPS and inconspicuously 'flagged' using natural markers (e.g. rocks, branches placed near the nests) rather than a stake or flag that could attract visual predators including Pied Crows (Picozzi 1975; Bogliani & Bellinato 1998; Rollinson & Brooks 2007). Latex gloves were worn when handling eggs and conducting all associated work at these nest sites in order to minimize human scent which could attract or deter non-avian predators (MaCivor et al. 1990; Zanette 2002). One plot was created per day, and the order of plot setup was random.

Artificial nests (Fig. 2d — f) were created to match the density and appearance of natural Kittlitz's Plover nests (Fig. 2a — c) as closely as possible whilst still maintaining egg visibility. Nests were placed at least 20 m apart in suitably open microsites (i.e. not concealed under a bush or tree) and the scrapes were dug approximately 3 cm deep and 11 cm wide using a steel spoon. Two Japanese quail (*Coturnix japonica*) eggs were placed in each scrape to match average Kittlitz's Plover clutch size (Hockey et al. 2005). Once in the scrape eggs were only partially covered (50%) with soil as covering them completely would likely have prevented predation almost completely (Appendix B). Real eggs were used in all phases because using 'fake' eggs (clay, plastcine or plastic) might deter predators from predating subsequent nests at a site, thereby preventing assessment of the effect of carbachol on nest predation. Quail eggs have been used in other studies as a suitable mimic for numerous ground-nesting birds for example Piping Plover (*Charadrius melodus*) (MaCivor et al. 1990), California Least Tern (*Sterna antillarum*) (Avery et al. 1995) and Northern Lapwing (*Vanellus vanellus*) (Šálek & Zámečník

2014). Although quail eggs can vary substantially from one to another in size, shape, colour and patterning and likely differ from Kittlitz's Plover eggs in some respects (Fig. 2, Appendix A), they are the closest match to Kittlitz's Plover eggs commercially available in South Africa. As such they represent an appropriate choice for testing CFA as a conservation management tool. Eggs were sourced from a local supplier (SA Quail Breeders, Rockliff Farm, East London at R3.30 or \$0.21 per egg) and were kept refrigerated until they were provisioned within 3 weeks for untreated eggs and within 4 days after processing for treated eggs (Avery et al. 1995; Cox et al. 2004).

## ***ii) Data collection***

Artificial nests were checked every two days for signs of nest predation or other damage. For each nest check, nests were recorded as 'intact', defined as both eggs remaining in scrape, or 'damaged', defined as either one or both eggs missing, pecked, broken or displaced up to 3 m away from the nest location (Fenske-Crawford & Niemi 1997). Photographs were taken of every damaged nest for future reference. Where possible, any other signs of predation such as animal tracks, feathers or fur visible within a 3 m radius of a damaged nest (Dimmick & Nicolaus 1990) were also noted and photographed. To avoid any attraction or deterrence of predators from plots and nests due to observer presence, minimal time was spent creating and checking individual nests (maximum 2 minutes) and where possible these were only done if no Pied Crows were visible within the plot or within the 100 m buffer zone from plot edges. Due to the time needed to travel between plots and to check nests in each site, it was only possible to check a maximum of 3 sites per day. Checking every other day may nevertheless be preferable than daily checks which could influence predator behavior (Salathe 1987).

Due to logistical problems, two of the sites were not checked with consistent 2 day intervals for all checks: Site A, 3 day interval between check 6 and 7 of treatment phase and Site F, 3 day interval between setup and check 1 of treatment phase and 2 day intervals thereafter. However in both cases it seemed justifiable to consider these as normal checks to allow for ease of analysis. For site A, the 7th check was conducted as early as possible on the morning of the third day at which point 4 out of 6 available nests were predated. Camera trap footage for one

of these nests revealed that the eggs had been predated 2 days prior and therefore had the check been conducted the day before according to protocol, the nest would have already been damaged. For site F, check 2 was similarly conducted as early as possible on the third day by which point 13/20 nests from check 1 had been predated. Given the high rate of predation recorded at the first check, it seems reasonable that most if not all of these nests were predated by the day when they should have been checked, with a small error introduced by the overnight delay. Consequently for the purpose of analyses, I did not differentiate these checks with three rather than two day intervals (2/126 total checks).

### ***Egg preparation***

A total of 360 quail eggs were processed for provisioning during the treatment phase. All the eggs were first emptied by piercing eggs at opposite ends, mixing the egg contents inside the shell using a dissecting needle to break internal membranes and then using a syringe or straw to blow air through one end and force the contents out the other. One end was resealed using hot-melt glue (Rapid EG212, 20W Glue Gun and Glue Sticks, Builders Warehouse, Cape Town, South Africa), the egg refilled using a 10 mm disposable syringe and the second hole sealed as before (Avery et al. 1995; Cox et al. 2004; Gabriel & Golightly 2011, 2014). Eggs were rinsed with water to remove any excess contents on the shell. 180 'neutral' eggs were re-filled with water, and 180 'emetic' eggs were re-filled with an egg-carbachol mixture.

The carbachol mixture placed in treatment eggs was made using a suitable amount of 100 mg/ml carbachol solution (0.31ml ~ 31 mg for every egg processed or 9 g of mixture made, see below for dosage determination). This solution was made up to the required volume of mixture (approximately 9 g for every egg processed) with the whisked egg contents and then mixed thoroughly. Carbachol (carbamoylcholine chloride  $\geq 98\%$  titration, crystalline, Sigma-Aldrich Pty Ltd, Kempton Park, South Africa at R5436.38 ~ \$342.59 per 10 g) was weighed on an electric scale (accurate to 0.1 g) and dissolved thoroughly with distilled water to make up the solution which was kept in a clearly labeled glass jar until use. Latex gloves and a laboratory coat were worn during egg preparation for safety and to avoid contamination. Eggs were kept in a



refrigerator and used within 4 days of processing (Cox et al. 2004). Eggs were transported in clearly marked and sealed containers and any treated egg remains or whole eggs that were not consumed by the end of the treatment phase, were retained in marked plastic bags and later disposed at the University of Cape Town in chemical waste disposal bins.

### ***Carbachol dosage***

Carbachol was chosen as the most appropriate drug for inducing CFA in this study for a number of reasons. Firstly it is a colourless, tasteless and odourless chemical and thus presumably undetectable in food such that any induced behavioural aversion would be due to an egg's visual appearance (Nicolaus et al. 1989; Gabriel & Golightly 2014). It is also water-soluble, making it easy to prepare and administer (Nicolaus 1987). Furthermore, carbachol has more consistently proven efficacy for reducing egg predation compared to other aversive agents such as topical methyl anthranilate, methiocarb (Avery & Decker 1994) and trimethacarb (Nicolaus et al. 1983) to name a few. Several studies have demonstrated that when administered at an appropriate dosage, carbachol can induce complete avoidance without causing long term damage or fatalities in corvids (Nicolaus et al. 1989; Cox et al. 2004; Gabriel & Golightly 2011). Determining an appropriate dosage is key to maintaining this balance. When treated with an optimally effective dosage that quickly induces illness, crows can learn to completely avoid treated eggs and subsequently avoid untreated eggs for longer (Bogliani & Bellinato 1998; Cox et al. 2004).

Given the time constraints of this study it was not possible to capture crows and conduct the laboratory tests required to determine an optimal effective dose suited specifically to Pied Crow. There was also uncertainty as to how many eggs and how much of the egg contents wild predators would consume. On the one hand, an individual could potentially eat multiple treated eggs, consuming multiple doses of tasteless carbachol within a short time frame that might be poisonous (Cox et al. 2004; Gabriel & Golightly 2014). On the other hand, it was possible that individuals might only consume part of the egg contents and therefore ingest a low dosage which may either cause a delay between ingestion and sickness, or not cause illness

at all. Either one of these could prevent the animal from making an association between illness and the food item. Given these uncertainties and constraints and in order to minimize potential overdosing, whilst still aiming for induced avoidance, I used a conservative carbachol dosage that was used successfully to induce CFA in Steller's Jays (Gabriel & Golightly 2011).

I followed the lowest effective dose identified in laboratory studies undertaken by Gabriel & Golightly (2014) on Steller's Jays (mean body mass = 115 g) of 0.77 mg carbachol / g of egg. In their study, this dosage effectively induced illness and reduced attacks on eggs of the same type as treated eggs even though birds often ate only part of the egg contents. The dosage concentration was scaled up to match the average mass of the Pied Crow (mean body mass = 520 g; Hockey et al 2005). This yielded an identified dosage of 3.48 mg / g of egg equal to 31.34 mg carbachol per quail egg (average mass = 9 g). This was higher than the minimum dosage (24 mg / egg) required to induce aversion in American Crows as identified by (Nicolaus et al. 1989) but was significantly lower than the of 22.01 mg / g of egg (equivalent to 198.09 mg / quail egg) optimal dosage identified by Cox et al. (2004) for the similarly sized Carrion Crow (*Corvus corone*). One individual died during this experiment, potentially as a result of overdosing and I wished to avoid this possibility.

The more conservative approach was deemed appropriate given i) the efficacy of lower doses as demonstrated by Gabriel & Golightly (2014), ii) that prior lab tests were not possible, and iii) the number of egg predators present was beyond my control. Additionally, other wild animals would have been exposed. However, it is worth noting that Pied Crow were the most likely known egg predator and had the lowest mass relative to other possible predator species found in this environment. Finally, carbachol is locally expensive (R5436.38 ~ \$342.59 per 10 g) and consequently a minimum effective dose was targeted to optimize the financial viability of this conservation management strategy.

### ***Natural nests***

Effort was made to find any natural Kittlitz's Plover nests between the 25<sup>th</sup> September 2015 and the 23<sup>rd</sup> November 2015. This was done to determine natural nest survival rate, assess whether these were comparable with those of artificial nests and evaluate whether natural nests are afforded any protection if CFA was successfully induced. In most cases nest finding involved observing adult birds that were kicking sand over the eggs, or displaying other anti-predator tactics as an observer approached either on foot or in a vehicle (Troscianko et al. in prep). Nests were marked with a GPS and checked every 1 to 6 days (usually every 2 days) until they were predated, abandoned or the eggs hatched. To reduce the risk of nest failure due to disturbance, no checks were done on particularly overcast, wet or windy days or if the ambient temperature was very high (>30°C). Nest state at discovery was recorded as 'laying' if there was only one egg in the nest upon discovery and two eggs at either the next check or a later check, or otherwise as 'incubating'.

Nest fate was defined as either 'abandoned', 'predated' or 'hatched'. Nests were considered 'abandoned' if no bird had been seen near the nest location for 3 consecutive checks but the eggs remained. Considering that Kittlitz's Plovers remove eggshell remains from the nest soon after the eggs have hatched (Hockey et al. 2005), a nest was only considered 'hatched' if a brooding adult and chick(s) could be found within a 30 m radius of the nest site or on camera trap footage where available. To reduce the chance that hatched nests were mistaken for predated nests, a nest was only recorded as 'predated' if the nest was empty and there was no sign of a brooding adult and chick(s) within 5 minutes of searching the area (up to 30 m from nest site) or on camera footage where available.

### ***Predator point counts***

To identify likely predators and estimate predation pressure, one 5 minute point count using binoculars was made on arrival for every site check. At each site check, the count was conducted from the same position along the plot edge (Freemark & Rogers 1991) where

visibility of the entire plot was the best. Only predators visible within the plot and up to an estimated 100 m of the plot boundary were included in the count. For each predator observed, the species, time of first observation, total number of sightings per count (i.e. lone individuals or groups of a given species) and total number of individuals per count were recorded. To avoid repeat sampling of the same individuals over the 5 minute period, I made one full 360° rotation, recording all the predators seen within the specified range (Freemark & Rogers 1991). Notice was taken of individuals or groups seen moving outside of the range as well as the direction of movement, and any individuals or groups seen re-entering the plot from the same direction were excluded from the count.

### ***Predator observations***

Predator observations were also made on an ad hoc basis during the time spent checking nests in a plot. These were used to provide further information about predator numbers at a finer scale than just presence-absence, predator identity, potential responses to the ingestion of carbachol, behavior and movement within and around the site (Dimmick & Nicolaus 1990). For each individual seen, record was taken of the time first seen, the species, estimated distance from the plot (<100 m or ≥100 m), lowest estimated height above ground and behavior displayed. Based on what the individual was doing when it was first sighted, behaviour was classified into one of three categories: 1) 'search' if the animal was foraging, defined looking down at the ground whilst flying or circling less than 50 m overhead, 2) 'pass' if the animal was merely flying through, past or over the site without its head pointed down at the ground or if it was flying higher than 50 m above ground and 3) other (circling overhead, perched, sitting on the ground, feeding, aggressive and social interactions).

### ***Camera traps***

Nest cameras offer a more accurate and unbiased means of identifying predators and have been previously used at wader nests (Macdonald & Bolton 2008; Ekanayake et al. 2015).

Motion sensitive trail cameras (LTL Acorn 5310WVG programmable, high-resolution 940 nm MMS camera trap and ScoutGuard SG550V 5MP ultra compact digital scouting camera) were set up at artificial nests (2 to 3 cameras per plot) and as many natural nests as possible to identify egg predators and increase the chances of detecting more elusive or nocturnal predators (Macdonald & Bolton 2008) such as caracal, jackal, mongooses, bat-eared foxes, clawless otters and feral cats. A total of 49 cameras were deployed: 40 at artificial nests and 9 at natural nests. Cameras recorded during both the day and night. Each was placed 1 to 3 metres away from the nest (Hernandez et al. 1997) and nearby vegetation, rocks and blocks of dry mud were used to secure and conceal the cameras as much as possible without obscuring the field of view. Any vegetation in view that may have caused false triggering of the camera was removed or flattened without causing excessive disturbance of the nest sites. Cameras were programmed to take either 2 or 3 photographs (5 MP resolution) per trigger with a 5 second trigger interval and the LTL Acorn models were set to record 10 second videos of size 320x240 when triggered.

Camera placement was done as quickly as possible (maximum 5 minutes) especially at natural nests to minimise disturbance to the incubating adults. Natural nests were observed from a distance for a few minutes after camera placement to ensure that the bird returned to the nest. All cameras were checked on the second day after placement to ensure that they were functioning properly and not prone to false triggers. Batteries and memory cards were checked every 2 to 6 days thereafter and replaced as required. To reduce associative learning by predators that identify nest locations by spotting cameras (Ekanayake et al. 2015), two or three 'dummy cameras' (plastic boxes of the same size, colour and shape as real cameras) were placed at random within or just outside each plot.

Camera trap footage was later examined to identify predators (Ekanayake et al. 2015). An animal caught on camera, either in a photograph or video footage, was identified as the predator if the footage revealed the animal with an egg in its mouth or beak, pecking at the nest location or eating one or both eggs. In less obvious cases, even if a predator did not fulfill the above criteria, predator status was assigned if the potential predator was caught on camera

during the time between the check when the nest was found damaged and the previous check when it was still intact. The date and time of each predation event was recorded.

## **DATA ANALYSIS**

All analyses were conducted using R (v. 3.0.2, R Core Team, Vienna, AT) and the required software packages specific for each analysis. Generalized Linear Mixed Models (GLMMs) were fitted where necessary because these allow for the inclusion of random terms to control for repeated measures (Bolker et al. 2009). To assess presence-absence data and probabilities, models were fitted with a binomial distribution (logit link function) using the package 'lme4' (Bates et al. 2014). Models used to analyse count data were fitted with either a Poisson distribution (log link function) using the package 'lme4' (Bates et al. 2014), or where necessary a negative binomial distribution (logit link function) to account for zero-inflated or over-dispersed data using the package 'glmmADMB' (Skaug et al. 2015). Pearson residuals were used to evaluate over-dispersion where a ratio greater than 1 indicated over-dispersion. An information-theoretic approach using comparisons of Aikake's Information Criterion (AIC) was used for final model selection (Bolker et al. 2009), where the lower the AIC value, the better the model fit. An alpha  $p < 0.05$  indicated significance.

### ***Nest survival:***

#### ***i) Pre versus Post-treatment***

Overall nest survival probability to the end of a phase was very low during both the pre-treatment and post-treatment phases, such that the sample size of surviving nests was too low for a binomial analysis. Therefore to test for any measurable effect of treatment for increasing nest survival, a more fine scale analysis was conducted to assess this on a per check basis. Nest survival data from the pre and post-treatment periods ( $n = 240$  nests) were analysed in a

Poisson GLMM with the number of checks each nest survived as the response variable, and phase (pre-treatment, post-treatment) and treatment type (carbachol, water) as the explanatory variables with a fitted interaction term between these variables. The presence of a camera trap (presence, absence) was added as an extra explanatory variable to assess the effect of camera traps on nest survival. Site was included as a random factor to account for repeated measures. Following model selection, the model took the form:

```
glmer(checks.survived~phase*treatment+camera+(1|site),family=poisson)
```

To account for the changing density of available nests and any associated change in nest detectability, a further analysis investigating the likelihood of nest survival between checks was conducted. The number of nests surviving relative to the number of nests lost per check ( $n = 46$  checks, checks where no nests previously survived were excluded) was the proportional response variable considered in the binomial GLMM. Treatment type (carbachol, water) and phase (pre-treatment, post-treatment) were once again the explanatory variables considered, but in addition to site, check number was added to this model as a random effect. To account for the changes in nest density over time, the number of available nests (those intact at the previous check) was added as an offset variable although the effect this had on predation was relaxed by using log transformed values. After model selection, the random model took the form:

```
glmer(y~treatment*phase+offset(log(previous.nest.total))+(1|site)+(1|site.check),  
family=binomial)
```

where  $y$  was the proportional response variable generated using the `cbind` function in R and took the general form ‘number of successes: number of failures’ (Crawley 2007), in this case ‘the number nests survived: number nests lost’ on a per check basis. This response was simply the likelihood of nest survival between checks.

To provide a standard measure of nest survival, Daily Survival Rates ( $DSR \pm 1$  S.D) overall and per site were calculated for nests during pre-treatment and post-treatment. Mayfield’s method (Mayfield 1975) was applied as described by Jehle et al. (2004), such that:

$$DSR = 1 - \frac{\text{no. of failed nests}}{\text{no. of exposure days}}$$

where the 'no. of exposure days' is the number of days between when the nest was found or created and the midpoint between the last two observations. The standard deviation of daily survival rate was calculated as follows:

$$SD = \sqrt{\frac{(\text{no. of exposure days} - \text{no. of failed nests}) \times \text{no. of failures}}{\text{no. of risk days}^3}}$$

### **ii) Treatment phase**

To assess for any changes in the carbachol effect over time, a further binomial GLMM analysis was conducted using only treatment phase nest data for the number of nests surviving relative to the number of nests lost per check ( $n = 40$  checks, checks where no nests previously survived were excluded). This data was analysed separately from that comparing pre and post-treatment phases, because the number of artificial nests during the treatment phase (30 nests per site) was greater than that in the pre and post-treatment phase (20 nests per site). To test for any differences in the likelihood of nest survival as exposure time to either water or carbachol-treated eggs increased, check number was added to the model as an explanatory term interacted with treatment type. Using the proportional response variable ( $y = \text{the number nests survived} : \text{number nests lost}$ ), allowed for the inclusion of replacement nests in the analysis even though these nests were created at staggered time intervals. The final model took the form:

$$\text{glmer}(y \sim \text{treatment.type} * \text{check.number} + \text{offset}(\log(\text{previous.nest.total})) + (1 | \text{site}), \\ \text{family} = \text{binomial})$$

To investigate the interaction term, data used for the above analysis was subsequently split according to treatment type and these datasets analysed separately to test whether different treatment types produced a different effect ( $n = 19$  checks for carbachol-treatment,  $n = 21$  checks for water-treatment). Both these binomial GLMMs took the form:



glmer(y~check.number+offset(log(previous.nest.total))+(1|site),family=binomial)

### ***iii) Post-treatment phase***

Due to a small sample size of surviving nests at water-treated sites during the post-treatment phase, it was not possible to conduct a similar analysis using post-treatment phase data to test for the latency of the carbachol effect. However, descriptive statistics are provided regarding the cumulative number of nests lost as time progressed during this phase.

### ***iv) Natural versus Artificial nests***

A comparison of natural and artificial nest survival rates is useful for assessing artificial nest predation as an indicator of what might happen to natural nests as a result of treatment. Specifically, where patterns of nest predation on artificial eggs reflect those on natural eggs, we may be reasonably confident that observations of predation on artificial eggs are a good qualitative proxy for predation on natural nests. To determine whether using CFA can protect natural plover nests one would ultimately need to identify whether natural nests in or near carbachol-treated plots show increased survival rates after treatment whereas those in or near water-treated plots show no change after treatment. Unfortunately in this study, it was not possible to make pre-treatment versus post-treatment comparisons for natural nest survival given that only three Kittlitz's Plover nests were found and monitored during post-treatment. However it was possible to compare natural nest survival with artificial nest survival during the pre-treatment period alone ( $n = 16$  natural nests,  $n = 100$  artificial nests excluding the artificial nests at site C given that there were no natural nests found there). Daily nest survival rates ( $DSR \pm 1$  S.D) were calculated for each nest type overall and per site using Mayfield's estimator as described above. Mayfield's estimator was chosen as the most appropriate measure because it assumes constant survival rates independent of nest stage and therefore does not require that nest stage be determined in the field (Jehle et al. 2004). Absolute survival rates of artificial nests was expected to be lower than for natural nests (Zanette 2002). Therefore DSRs were plotted across sites to identify any patterns in survival rate which likely reflect patterns in

predation rate (Zanette 2002) and to assess if the relative patterns were similar for both nest types.

***Predators:***

***i) Pre versus Post-treatment: Point counts***

Given that only Pied Crows were caught damaging nests on the camera traps (see results), only data for crows was considered for the predator point count and sighting rate analyses. To test if carbachol treatment reduced the likelihood of seeing a crow, I used a GLMM with the binomial response variable crow presence absence during a point count ( $n = 96$  point counts) with treatment phase (pre-treatment, post-treatment) and treatment type (water, carbachol) as explanatory factors, and site as a random factor. The interaction between phase and treatment type was fitted. The final model took the form:

```
glmer(crow.binomial~phase*treatment.type+(1|site),family=binomial)
```

***ii) Pre versus Post-treatment: Sighting rate***

To investigate whether the number of Pied Crow sightings per site check varied with treatment type and phase, a Poisson GLMM was run using the count of crows seen during a site check as the response variable ( $n = 83$  checks as adverse weather conditions prevented an adequate site check at site B on one day during the post-treatment phase). To account for the duration of the observation period, the time spent in the site ( $\log^{10}$  transformed for Poisson distribution) was added as an offset variable (logminutes). The explanatory and random factors were the same as for the point count analysis above. Site checks where more nests required checking might result in sighting rate bias and data usage was consequently restricted to observations of crows that were 'searching' in or 'passing' through, past or over the plot (as defined in the field methodology) and to conspicuous individuals seen within 100 m of the boundary. The final model took the form:

```
glmer(crow.total~phase*treatment+(1|site)+offset(logminutes),family=poisson)
```

**ii) Treatment phase: point counts and sighting rate**

Two further analyses were conducted using only treatment phase predator data to assess whether crow visitation rates varied with exposure time to either water or carbachol treated eggs. Check number was added to both models as an explanatory term interacted with treatment type, but the interaction term was subsequently dropped from the models as it had a non-significant effect on the respective response variables ( $p > 0.05$ ). Crow visitation rates during the pre-treatment phase at each check were accounted for by adding these to the models as offset variables. This allowed for the comparison of treatments having controlled for differential background predation rates in the different sites which could confound the treatment effect. Controlling for background predation rates in this way was not necessary for the pre versus post-treatment comparison because these phases could be compared directly to determine treatment effects and effects of site differences were adequately accounted for through the addition of site as a random effect. The two analyses were:

- (a) Binomial GLMM using crow presence absence data during a point count ( $n = 48$  counts) as the response variable offset against pre-treatment crow presence absence per count (crow.binomial\_pre). Following model selection, the final model took the form:

```
glmer(crow.binomial~treatment+check.number+(1|site)+offset(crow.binomial_pre),  
      family=binomial)
```

- (b) Poisson GLMM using the number of crow sightings during a site check ( $n = 42$  checks) as the response variable offset against pre-treatment crow sighting rate ( $\log^{10}(1+\text{no.of crows/minute})$ ) and the length of observation of the treatment phase site check (logminutes). Following model selection, the final model took the form:

```
glmer(crow.total~treatment+check.number+(1|site)+offset(logminutes)  
      +offset(log(1+no.ofcrows/minute),family=poisson)
```

## RESULTS

### *Nest survival:*

#### *i) Pre versus Post-treatment*

Overall nest survival probability to the end of a phase (14 days) was very low for both the pre-treatment and post-treatment phase. Only 1/120 nests (site E) survived to the end of the pre-treatment phase and only 4/120 nests (carbachol-treated site F) survived to the end of the post-treatment phase. A more detailed investigation of nest survival in terms of number of checks survived using a Poisson GLMM revealed that the phase:treatment type interaction was significant and this was driven by increased survival at carbachol-treated plots ( $Z = -4.941$ ,  $p < 0.001$ ,  $n = 240$  nests; Table 1a, Fig. 4a). Nest survival rates were similar in all plots pre-treatment but during post-treatment, nests survived longer in carbachol plots whereas they survived less time in water-treated plots. Nests survived almost twice as many checks after carbachol treatment compared to before (pre: 0.9 [+ 0.84: - 0.42], post: 1.6 [+ 1.50: - 0.76]; Fig. 4a). In contrast, nests survived almost four times fewer checks post-treatment with water compared to pre-treatment (pre: 0.3 [+ 0.33: - 0.16], post: 0.1 [+ 0.09: - 0.04]). The presence of a camera had no effect on nest survival ( $Z = 0.931$ ,  $p = 0.352$ ; Table 1a) and therefore this factor was disregarded in all subsequent analyses.

Similarly, results from the binomial GLMM analysis investigating the likelihood of nest survival between checks showed that the phase:treatment type interaction was significant ( $Z = -3.710$ ,  $p < 0.001$ ; Table 1b, Fig. 4b). This shows decreased survival of nests between checks following water treatment, in contrast with increased survival following carbachol treatment (Fig. 4b). The likelihood of nest survival between checks following water treatment was four and a half times lower than before treatment (pre: 0.024 [+ 0.0360: - 0.0148], post: 0.005 [+ 0.0092: - 0.0034]) whereas the likelihood of nest survival between checks was more than one and a half times higher following carbachol treatment relative to before (pre: 0.069 [+ 0.0826: - 0.0393], post: 0.109 [+ 0.1159: - 0.0599]).

Table 1. Summary results of a) a Poisson GLMM investigating the number of checks survived by nests, and b) a binomial GLMM investigating the likelihood of nest survival between checks for artificial plover nests created during the CFA trial. ( $n = 240$  nests: 6 sites, 2 phases, 20 nests per site per phase).

Model term	Level	Effect $\pm$ S.E.	Z	P
a) Poisson GLMM investigating the number of checks survived				
Intercept		- 1.23 $\pm$ 0.750	-1.63	0.102
Phase	Post-treatment	-1.38 $\pm$ 0.370	-3.72	<b>&lt; 0.001</b>
Treatment type	Carbachol	1.04 $\pm$ 1.011	1.032	0.302
Camera	Yes	0.19 $\pm$ 0.200	0.931	0.352
Phase:Treatment type	Post-treatment:Carbachol	1.98 $\pm$ 0.401	4.941	<b>&lt; 0.001</b>
b) binomial GLMM investigating the likelihood of nest survival between checks				
Intercept		-3.69 $\pm$ 0.944	-3.91	<b>&lt; 0.001</b>
Phase	Post-treatment	-1.55 $\pm$ 0.472	-3.28	<b>0.001</b>
Treatment type	Carbachol	1.09 $\pm$ 1.125	0.972	0.331
Phase:Treatment type	Post-treatment:Carbachol	2.04 $\pm$ 0.550	3.710	<b>&lt; 0.001</b>

Significant  $p$  values are in **bold**

† Pre-treatment, carbachol was the reference category

†† Site was a random effect in the model

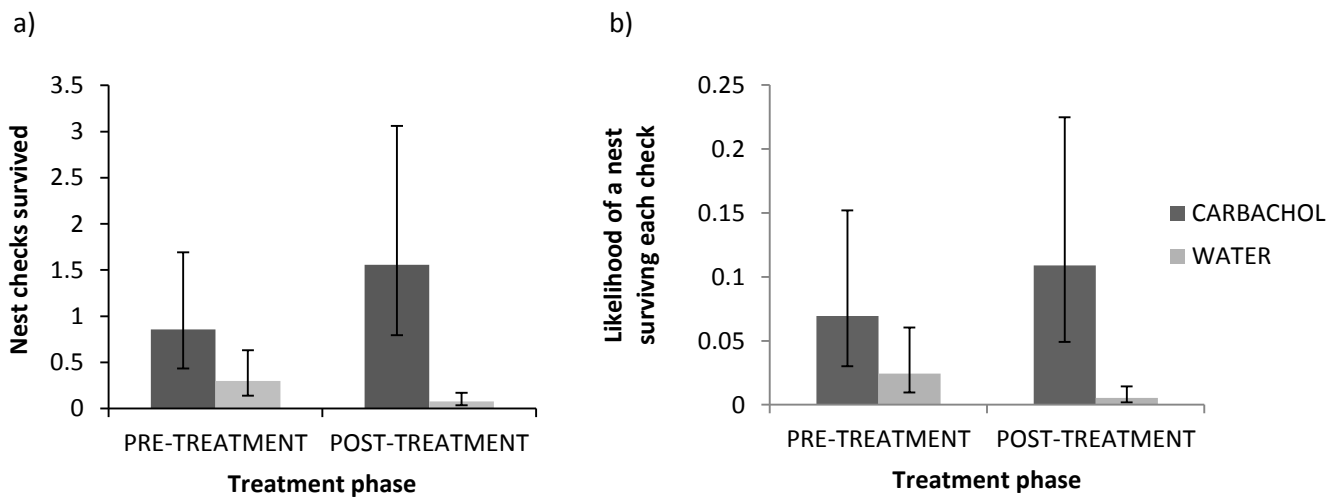


Figure 4. GLMMs showing a) the number of checks survived by nests and b) the likelihood of nest survival between checks, for artificial plover nests created during the CFA trial. (Values are given as a back-transformed mean  $\pm$  S.E.,  $n = 240$  nests; 6 sites, 2 phases, 20 nests per site per phase).

As a standard measure of survival the average daily survival rate (DSR  $\pm$  1 SD) of artificial nests post-treatment with carbachol was 15% higher compared to before treatment (pre:  $0.71 \pm 0.032$ , post:  $0.81 \pm 0.023$ ; Fig. 5). The opposite was true in water-treated sites, where the nest survival rate was 40% lower after treatment than before treatment (pre:  $0.54 \pm 0.044$ , post:  $0.22 \pm 0.047$ ).

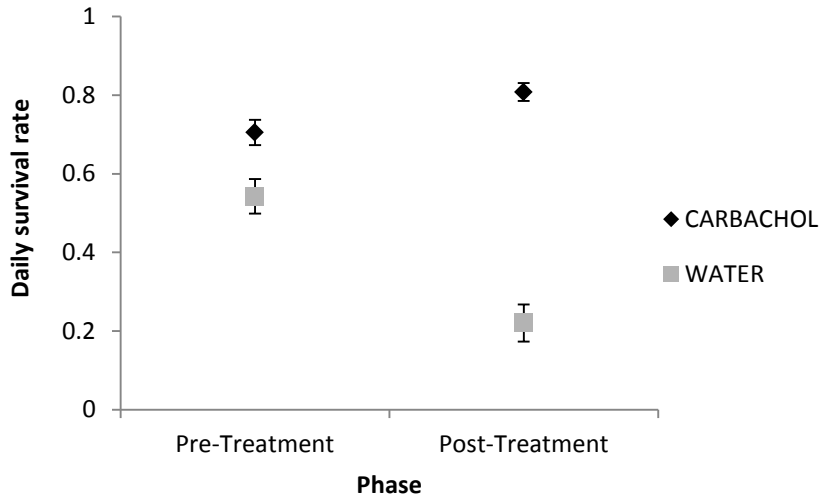


Figure 5. Mayfield's estimates of daily survival rate (DSR  $\pm$  SD) calculated for artificial nests monitored during the CFA trial in 6 independent sites. DSR was higher in carbachol sites after treatment (60 nests, 56 failures, 292 exposure days) but lower in water sites after treatment (60 nests, 60 failures, 77 exposure days) relative to before.

### ***ii) Treatment phase***

Binomial GLMM analysis of the likelihood of nest survival between checks during the treatment phase revealed that this changed significantly over time and that the trend differed according to treatment type (significant treatment type:check number interaction:  $Z = 4.129$ ,  $p < 0.001$ ; Table 2a, Fig. 6). Further separate analyses of data from the two treatment types showed that this significant effect was driven principally by significant increases in nest survival over time at carbachol-treated sites (check number :  $Z = 0.42$ ,  $p < 0.001$ ; Table 2b). By the end of the treatment phase, the likelihood of nest survival between checks at these sites had increased

over eight-fold (check 1: 0.04 [+ 0.014: - 0.010], check 7: 0.32 [+ 0.114: - 0.096]). In contrast, the likelihood of nest survival between checks decreased almost three-fold at water-treated sites (check 1: 0.06 [+ 0.054: - 0.030], check 7: 0.02 [+0.026: - 0.011]) although this change was non-significant (check number:  $Z = -0.19$ ,  $p = 0.082$ ; Table 2c).

Table 2. Summary results of binomial GLMMs investigating the likelihood of nests surviving between checks according to a) treatment type and check number ( $n = 120$  nests), b) check number (carbachol treatment only,  $n = 60$  nests) and c) check number (water treatment only,  $n = 60$  nests) for artificial nests created during the CFA trial.

Model term	Level	Effect $\pm$ S.E.	Z	P
a) effect of treatment type and check number				
Intercept		-2.53 $\pm$ 0.578	-4.379	< <b>0.001</b>
Treatment type	Carbachol	-1.16 $\pm$ 0.809	-1.436	0.151
Check number		0.18 $\pm$ 0.110	-1.596	0.110
Treatment type:Check number	Carbachol	0.58 $\pm$ 0.141	4.129	< <b>0.001</b>
b) effect of check number on likelihood of carbachol-treated nests surviving between checks				
Intercept		-3.69 $\pm$ 0.387	-9.538	< <b>0.001</b>
Check number		0.42 $\pm$ 0.089	4.690	< <b>0.001</b>
c) effect of check number on likelihood of water-treated nests surviving between checks				
Intercept		-2.52 $\pm$ 0.721	-3.500	< <b>0.001</b>
Check number		-0.19 $\pm$ 0.112	-1.742	0.082

Significant  $p$  values are in **bold**

† Water was the reference category for model (a)

†† Site was a random effect in all models

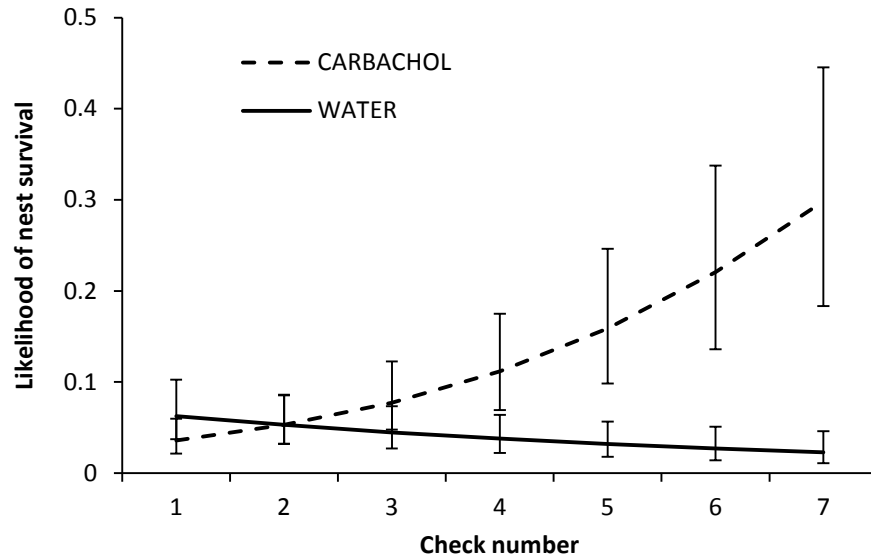


Figure 6. Binomial GLMM (treatment phase only), showing that the likelihood of artificial nest survival (back-transformed mean  $\pm$  S.E.) increased significantly with time (check number) in carbachol-treated sites ( $n = 60$  nests) whereas it decreased with time in water-treated sites, although non-significantly ( $n = 60$  nests).

### ***iii) Post-treatment phase***

During the post-treatment phase nests were lost rapidly from water sites, making a comparative analysis between the two treatment types (similar to that for the treatment phase described in ii) above) impossible due to a lack of data points for water-treated sites in later checks. On average, all nests were lost by the second check at water-treated sites (only one nest at one site survived more than 2 checks) whereas at carbachol sites on average only 11 out of 20 nests had been lost by the second check (Fig. 7). However, even though the rate of nest loss at carbachol-treated sites was slower than at water-treated sites, by the seventh and last check all nests at 2/3 carbachol-treated sites had been lost, and there were just 4 nests remaining at the third site.



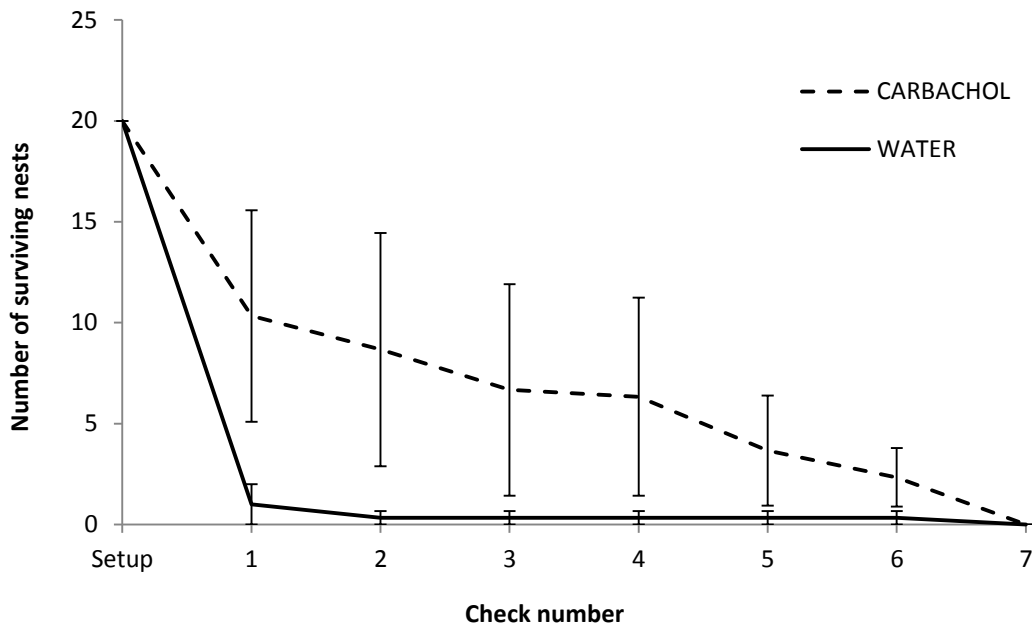


Figure 7. Number of artificial nests surviving each check (mean  $\pm$  S.E.) during the post-treatment phase of the CFA trial ( $n = 120$  nests; 60 nests per treatment type).

#### ***iv) Natural versus Artificial nests***

Prior to treatment, the average daily survival rate (DSR  $\pm$  1 SD) of natural nests ( $0.87 \pm 0.032$ ,  $n = 16$  nests, 14 failures, 109.5 exposure days) was approximately 25% higher than that of artificial nests ( $0.63 \pm 0.029$ ,  $n = 100$  nests, 99 failures, 270.5 exposure days; Table 3). Across sites, the general pattern of artificial nest survival was similar to that of natural nests; sites with a low artificial nest survival rate also had a low natural nest survival rate relative to other sites and vice versa (Fig. 8). The most notable exception was site D, which had the highest natural survival rate (DSR =  $1 \pm 0$ ,  $n = 1$  nest) but the lowest artificial survival rate (DSR =  $0 \pm 0$ ,  $n = 20$  nests), although only one natural nest was monitored at this site. Excluding site D, site A showed the highest daily survival rate for both artificial ( $0.81 \pm 0.037$ ) and natural nests ( $0.95 \pm 0.051$ ), whereas site F showed the lowest survival rate for both artificial ( $0.58 \pm 0.072$ ) and natural nests ( $0.78 \pm 0.196$ ). These results should be treated with caution given the small sample size of natural nests spread across sites.

Table 3. Summary of parameters used to calculate Mayfield's estimator of Daily Survival Rate (DSR  $\pm$  SD) for artificial and natural Kittlitz's Plover nests during the pre-treatment phase of the CFA trial.

Site	Natural			Artificial		
	Total n (failures)	No. of exposure days	DSR $\pm$ SD	Total n (failures)	No. of exposure days	DSR $\pm$ SD
A	2 (1)	19	0.95 $\pm$ 0.051	20 (20)	108	0.81 $\pm$ 0.037
B	8 (8)	53	0.85 $\pm$ 0.049	20 (20)	48	0.58 $\pm$ 0.071
C	n/a	n/a	n/a	n/a	n/a	n/a
D	1 (0)	14	1 $\pm$ 0	20 (20)	20	0 $\pm$ 0
E	4 (4)	19	0.79 $\pm$ 0.094	20 (19)	47	0.60 $\pm$ 0.072
F	1 (1)	4.5	0.78 $\pm$ 0.196	20 (20)	47.5	0.58 $\pm$ 0.072
Overall	16 (14)	109.5	0.87 $\pm$ 0.032	100 (99)	270.5	0.63 $\pm$ 0.029

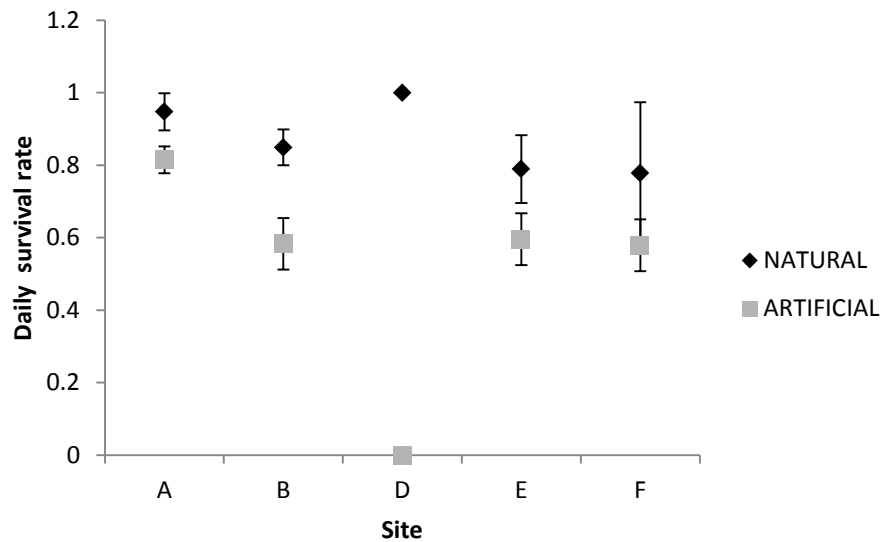


Figure 8. Mayfield's estimates of daily survival rate (DSR  $\pm$  SD) for natural Kittlitz's Plover nests ( $n = 16$  nests, 14 failures, 109.5 exposure days) and artificial nests ( $n = 100$  nests, 99 failures, 270.5 exposure days) monitored during the pre-treatment phase (14 days) of the CFA trial in 5 independent sites used for the CFA trial.

**Predators:**

**Camera trap footage analysis**

A total of 38 egg predation events, (33 at artificial nests and 5 at natural nests) were attributed to Pied Crow based on camera trap footage recorded during the study period (Table 4). Of these, 34 predators were ‘unambiguously’ identified as a Pied Crow (Appendix C), 30 at artificial nests and 4 at natural nests. At an additional 2 artificial nests, the potential predator was less easily identifiable from the photographs, but these ‘ambiguous’ predators were assumed to be crows on closer examination. In both cases the bird had black feathers and was approximately the same size as a Pied Crow. For one of the cases the photograph showed what appeared to be lower mandible of a cawing crow with puffed up throat, rather than the long beak of a Glossy Ibis (*Plegadis falcinellus*), the only other possibility. In the other case the legs were black rather than yellow like those of the Glossy Ibis. At a further 2 nests (1 artificial and 1 natural), although the initial cause of nest loss was ‘unknown’, in other words there was no footage of a potential predator, Pied Crow were recorded removing the second egg a few days later. Consequently all predation events observed in the camera footage were likely by Pied Crows.

Table 4. Camera trap footage analysis showing the total number of motion sensitive trail cameras deployed at artificial and natural Kittlitz’s Plover nests during the CFA trial, the number of predation events recorded and the number of times the predator was identified as a Pied Crow.

Phase	Artificial nests		Natural nests	
	No. of cameras	No. of predation events(all by Pied Crow)	No. of cameras	No. of predation events(all by Pied Crow)
Pre-treatment	13	11 (11)	n/a	n/a
Treatment	14	11 (11)	n/a	n/a
Post-treatment	13	11 (11)	n/a	n/a
Total	40	33 (33)	9	5 (5)

† No. of predation events included: 34 ‘unambiguous’ cases (predator identity was clear), 2 ‘ambiguous’ cases (predator was assumed to be Pied Crow based on general size and colour) and 2 ‘unknown’ cases (initial cause of nest loss unknown but a Pied Crow was recorded taking the second egg).

A nest camera was present at all 3 natural nests that hatched successfully. Footage from a camera placed near one of two nests that were eventually abandoned showed that the adult stopped incubating 3 days after the nest was found and revealed that the presence of the camera and or frequent disturbance from passing cars, humans and other birds such as Blacksmith Lapwing (*Vanellus armatus*) and Blue Crane (*Anthropoides paradiseus*) may have caused this.

### ***Direct predator observations***

During the entire experimental period, a total of 35 feeding events by predators were directly observed including 34 during site checks and 1 as an ad hoc behavioural observation of predation of a natural nest. All feeding events were by avian predators, and more than 50% of these were Pied Crow ( $n = 19$ ), whilst the rest were Kelp Gull (*Larus dominicanus*,  $n = 4$ ) and various raptors including Yellowbilled Kite (*Milvus aegyptius*,  $n = 4$ ), Osprey (*Pandion haliaetus*,  $n = 3$ ), Jackal Buzzard (*Buteo rufofuscus*,  $n = 3$ ), Rock Kestrel (*Falco rupicolus*,  $n = 1$ ) and African Marsh Harrier (*Circus ranivorus*,  $n = 1$ ). However, of all these feeding events, only Pied Crow ( $n = 10$ ) and Kelp Gull ( $n = 1$ ) were seen predating nests or eating what was definitely an egg. In conjunction with observations from nest cameras, these observations suggest that the Pied Crow is the key egg predator in this area.

### ***i) Pre versus Post-treatment: Point counts***

A total of 96 predator point counts were included in this analysis: pre-treatment phase  $n = 48$ , post-treatment  $n = 48$ . The binomial GLMM investigating the likelihood of sighting a Pied Crow during a 5 minute point count, revealed a near significant interaction between treatment type and phase ( $Z = - 1.808$ ,  $p = 0.071$ ; Table 5a, Fig. 9a) indicating that trends differed between treatment types. The likelihood of sighting a crow after carbachol treatment was half of that prior to treatment (pre: 0.21 [+ 0.099: - 0.073], post: 0.12 [+ 0.087: - 0.054]). In contrast, the

likelihood of seeing a crow after water treatment was three and a half times higher than before treatment (pre: 0.08 [+ 0.079: - 0.042], post: 0.29 [+ 0.106: - 0.087]).

**ii) Pre versus Post-treatment: Sighting rate**

A total of 83 site checks were included in this analysis (pre-treatment phase  $n = 42$ , post-treatment  $n = 41$  as adverse weather conditions prevented an adequate site check at site B on one day during the post-treatment phase). Observations made during plot setup site visits were excluded from this analysis as these could have introduced observer bias. The Poisson GLMM assessing crow visitation rate during a site check indicated a significant phase:treatment type interaction ( $Z = - 2.321$ ,  $p = 0.020$ ; Table 5b, Fig. 9b). At carbachol-treated sites the number of crow sightings per check during post-treatment was almost halved when compared to that during pre-treatment (pre: 0.14 [+ 0.055 - 0.039], post: 0.07 [+ 0.038: - 0.025]). The opposite trend was seen at water-treated sites where the number of crow sightings per check after treatment was twice as high as it was prior to treatment (pre: 0.07 [+ 0.036: - 0.024], post: 0.15 [+ 0.060: -0.042]).

Table 5. Summary results of a) a binomial GLMM investigating the likelihood of seeing at least one Pied Crow during a 5 minute point count and b) a Poisson GLMM investigating the number of crow sightings during a site check during the CFA trial ( $n = 96$  point counts, 83 checks).

Model term	Level	Effect ± S.E.	Z	P
a) binomial GLMM investigating the likelihood of seeing at least one Pied Crow per point count				
Intercept		-2.42 ± 0.762	-3.173	<b>0.002</b>
Phase	Post-treatment	1.52 ± 0.869	1.75	0.080
Treatment type	Carbachol	1.07 ± 0.914	1.172	0.241
Phase:Treatment type	Post-treatment:Carbachol	-2.14 ± 1.181	-1.808	0.071
b) Poisson GLMM investigating the number of crow sightings during a site check				
Intercept		-2.66 ± 0.419	-6.351	<b>&lt; 0.001</b>
Phase	Post-treatment	0.74 ± 0.430	1.712	0.087
Treatment type	Carbachol	0.69 ± 0.528	1.307	0.191
Phase:Treatment type	Post-treatment:Carbachol	-1.40 ± 0.602	-2.321	<b>0.020</b>

Significant  $p$  values are in **bold**

† Pre-treatment, carbachol was the reference category

†† Site was a random effect in both models, and length of observation (logminutes) was an offset variable in (b) only

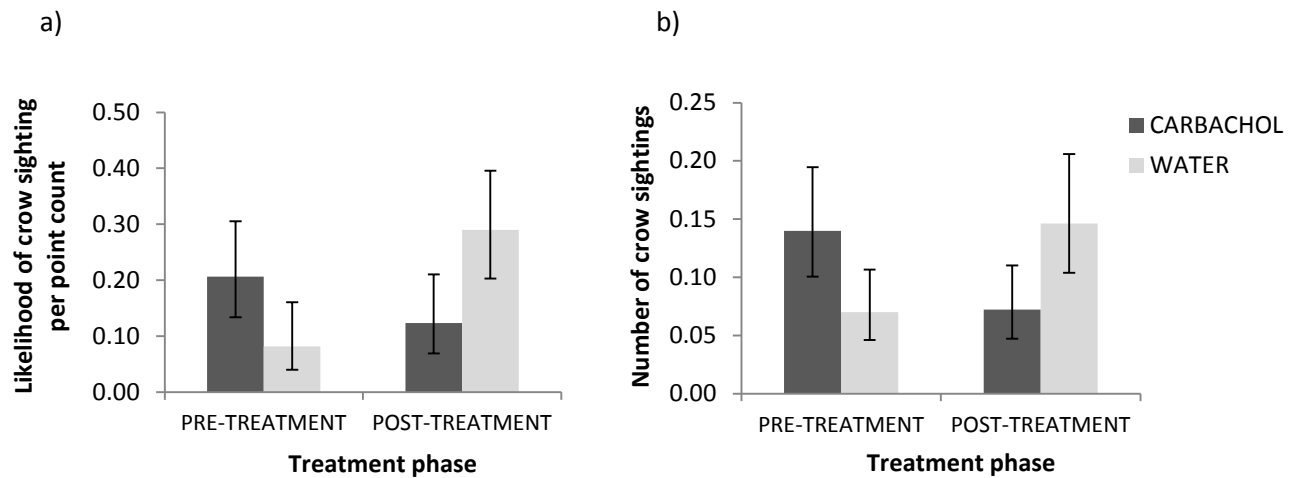


Figure 9. GLMMs showing a) the likelihood of sighting a Pied Crow during a 5 minute point count and b) the number of crow sightings during a site check (offset against observation time in minutes) during the CFA trial. (Values are given as a back-transformed mean  $\pm$  1 S.E.,  $n = 96$  point counts, 83 checks).

### iii) Treatment phase: point counts and sighting rate

(a) The binomial GLMM analysis revealed a non-significant checknumber:treatment type interaction ( $Z = -0.44$ ,  $p = 0.385$ ; Table 6a, Fig. 10a) indicating that carbachol-treated sites and water-treated sites showed similar trends over the course of the treatment phase in terms of the likelihood of seeing at least one Pied Crow during a treatment phase point count. Treatment type and check number were subsequently considered as separate fixed effects and treatment type was found to have a significant effect on the likelihood of seeing a crow ( $Z = -1.98$ ,  $p = 0.041$ ; Table 6a) but check number did not ( $Z = -0.28$ ,  $p = 0.135$ ; Table 6a). This indicated that overall, the likelihood of seeing at least one crow during a point count at carbachol-treated sites (mean range: 0.03 to 0.17) was lower than at water treated sites (mean range: 0.17 to 0.59; Fig. 10a).

(b) Similarly, the Poisson GLMM revealed a non-significant checknumber:treatment type interaction ( $Z = 0.03$ ,  $p = 0.864$ ; Table 6b, Fig. 10b) indicating that carbachol-treated sites and water-treated sites showed similar trends over time in terms of the number of crows seen during a treatment phase site check. However, in contrast to the point count analysis,

even after the interaction term was dropped from the model, neither treatment type nor check number were significant determinants of the number of crows sighted during a check ( $p < 0.05$ ; Table 6b, Fig. 10b).

Table 6. Summary results of a) a binomial GLMM investigating the likelihood of seeing at least one Pied Crow during a 5 minute point count and b) a Poisson GLMM investigating the number of crow sightings during a site check during the treatment phase of the CFA trial ( $n = 48$  point counts, 42 checks).

Model term	Level	Effect $\pm$ S.E.	Z	P
a) binomial GLMM investigating the likelihood of seeing at least one Pied Crow per point count				
Intercept		0.37 $\pm$ 0.911	0.405	0.686
Treatment type	Carbachol	-1.98 $\pm$ 0.968	-2.045	<b>0.041</b>
Check number		0.28 $\pm$ 0.186	-1.496	0.135
Treatment type:Check number	Carbachol	-0.44 $\pm$ 0.511	-0.869	0.385
b) Poisson GLMM investigating the number of crow sightings during a site check				
Intercept		-1.73 $\pm$ 0.488	-3.545	<b>&lt; 0.001</b>
Treatment type	Carbachol	-0.13 $\pm$ 0.550	-0.235	0.814
Check number		-0.14 $\pm$ 0.093	-1.505	0.132
Treatment type:Check number	Carbachol	0.03 $\pm$ 0.186	0.171	0.864

Significant  $p$  values are in **bold**

† Water was the reference category in both models

†† Site was a random effect in both models

††† Offset variables: (a) pre-treatment crow presence (1/0) per count, and (b) pre-treatment crow sighting rate ( $\log(1+\text{no.ofcrows}/\text{hour})$ ) and the length of observation time ( $\log(\text{minutes})$ )

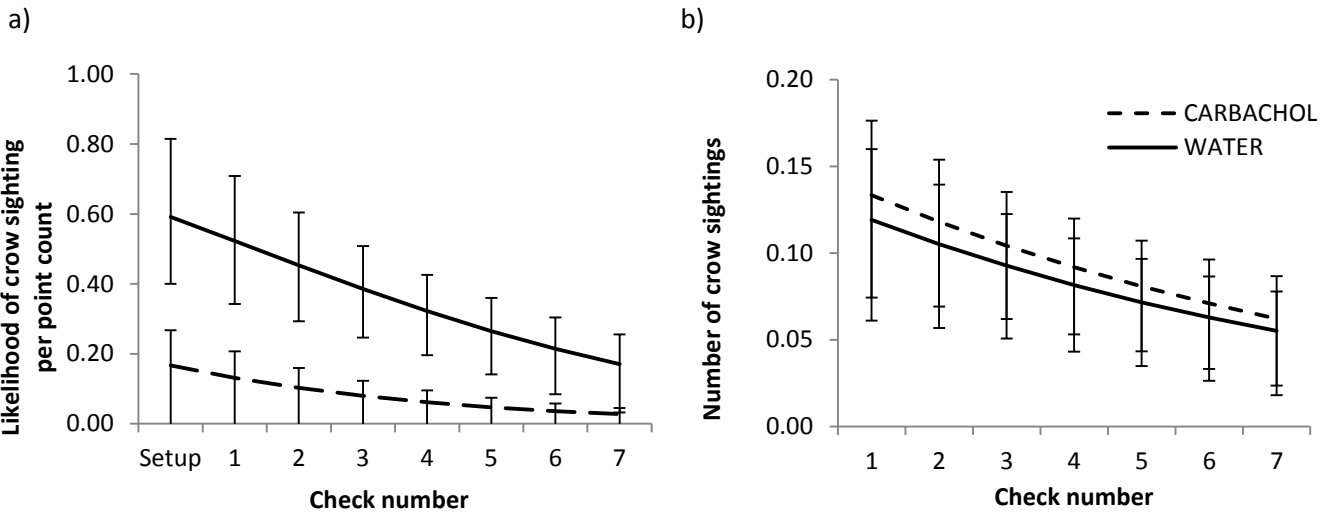


Figure 10. GLMMs (treatment phase only) showing a) the likelihood of sighting at least one Pied Crow during a 5 minute point count (offset against pre-treatment likelihood of sighting a crow) and b) the number of crow sightings during a site check (offset against observation time and pre-treatment crow sighting rate) during the CFA trial (Values are given as a back-transformed mean  $\pm$  1 S.E., ( $n$  = 48 point counts, 42 checks).



## **DISCUSSION**

This study indicates that CFA using carbachol as an aversive-agent can effectively deter nest predators, primarily the Pied Crow, from artificial plover nests in a natural environment. Treatment with carbachol-laced quail eggs was associated with considerably reduced predation rates on artificial nests, as evidenced by higher nest survival after carbachol treatment than before, and after carbachol treatment than after water treatment. Additionally nest predation rates declined during exposure periods with carbachol, but did not do so during exposure to water-filled eggs. Direct observations as well as camera trap footage confirmed that Pied Crow were the primary predators of both artificial nests and natural Kittlitz's Plover nests. Furthermore, Pied Crow were less frequently seen at sites following carbachol treatment, while the opposite was true at water-treated sites. Consequently patterns of reduced nest predation following exposure to carbachol appear to be driven by changes in predator behaviour indicative of CFA having been induced. However, predation of artificial nests was not completely eliminated after treatment and while natural nests at sites showed similar patterns of predation to artificial nests prior to experimental treatment, too few natural nests were available following treatment to assess whether these were afforded protection. As a result, whether CFA can effectively reduce predation of natural nests remains unclear. Results therefore provide evidence that CFA has the potential to be a valuable management tool in reducing nest predation on threatened birds, but further research is required to improve the efficacy of this technique and hone its use in conservation management.

### ***Provisioning emetic eggs to increase nest survival***

These findings suggest that a 14 day conditioning phase is sufficient time to induce some level of aversion in Pied Crows in agreement with CFA tests on other species of crow (American Crows, Dimmick & Nicolaus 1990; Carrion Crows, Cox et al. 2004). Nicolaus et al. (1989) found that after a conditioning phase with carbachol-laced chicken eggs, American Crows consumed five times fewer eggs in treatment sites compared to in control sites. However, the results may have been influenced by site-level differences in predation rate as these were not controlled for in this study. This can only be achieved through within site comparisons of pre-treatment

predation rates with treatment or post-treatment rates. Conover (1990) used a similar experimental design to mine incorporating three experimental phases and found that raccoon predation on artificial eggs was suppressed by more than 20% after eggs treated with emetine had been distributed. However, his study lacked an appropriate non-emetic control to rule out the possibility that changes in predation between phases may have been due to temporal variation in predation rather than the treatment itself. My study has improved on studies such as this through the combination of both geographically separate treatment and control sites whilst at the same time accounting for site-level differences by comparing predation during a pre-treatment period to that during a post-treatment period. In this way, the effects of treatment are isolated and site-level differences in predation rate are adequately accounted for. Consequently the results obtained are more robust and the effects seen can be attributed to CFA treatment with greater certainty.

Although my study and others suggest that CFA may be used to reduce nest predation, the possibility that egg provisioning can increase nest predation is a significant concern that has previously been overlooked. Within water-treated sites both crow visitation rates and artificial nest-predation rates were higher after treatment compared to before, suggesting that provisioning untreated eggs during the pre-treatment phase increases predation. Predation rates on untreated eggs have been shown to increase over time in this way before (Conover 1990). This indicates that if CFA is not successfully induced, that egg provisioning in a natural area could run the risk of boosting predation within sites. It is worth noting that predation rates at water-treated sites remained relatively consistent during the treatment phase suggesting that using non-rewarding eggs injected with water was appropriate. However, this result stands in contrast to those of Price and Banks (2012) who spread bird nest odours prior to provisioning artificial nests, thereby training black rats to ignore this unrewarding cue resulting in reduced nest predation. This unrewarding cue is equivalent to the water-filled egg control treatment in my study suggesting that this technique may not always be effective. However, in my study, it is more likely that the provision of rewarding eggs before and after unrewarding eggs was the reason for the elevated predation rates observed.

The increased predation at control sites after treatment compared to before could suggest that resident crows in the vicinity had learnt during the pre-treatment phase that these sites were productive foraging areas. Alternatively crows that were deterred from carbachol-treated sites may have been attracted to these sites. Whilst effort was made to ensure that sites were independent without compromising on sample size, it is possible that crows may have travelled between the sites. This distance between sites should be increased if possible to a distance informed by improved information on Pied Crow range size. Capture and ringing of individuals is also recommended to determine if sites are independent.

A key goal in tests of CFA is to determine whether provisioning emetic foods transfers protection to natural threatened species or their eggs. In this study it was not possible to explore whether natural nests benefit at all from nearby provisioning of carbachol-laced quail eggs. Nevertheless, overall predation rates and patterns of predation between sites and between artificial and natural nests were found to be similar. These comparative predation rates permit some confidence that my study of artificial nest predation qualitatively reflects the patterns that might be observed if manipulations were undertaken on natural eggs in natural nests rather than on quail eggs in artificial nests. Consequently, increased survival of natural nests following emetic egg provisioning may be predicted.

It is worth noting that in real terms, average artificial nest survival only increased from 1.8 days to 3.2 days as a result of treatment. Assuming that the artificial nests created here closely resemble natural nests, and that natural nests would benefit from a similar degree of protection, such a small increase in survival is unlikely to be biologically significant. ‘Predated’ natural nests only survived a maximum of approximately 13 days ( $n = 7$  ‘predated’ nests found during ‘laying’) and survival would need to be increased to between 22 and 28 days to reach the end of incubation (Hockey et al. 2005) to be worthwhile. These assumptions may not hold and further investigation is required to elucidate if and how natural nests would be protected, using spatio-temporal patterns of survival for both nest types as a guide. Furthermore, modifications to emetic egg provisioning techniques as discussed below, have the potential to greatly improve any effect emetic eggs have on nest survival.

Given that the overall aim of using CFA as a management tool is to protect natural nests from predation, high priority must be given to improve the similarity between artificial and natural nest appearance. Additionally, it is essential for future studies to detect and assess any effects on natural nests. Natural nests are unlikely to receive any benefit from CFA if they are easily distinguishable from artificial nests by predators (Conover & Lyons 2003). The ability of predators to distinguish natural nests from artificial nests, which is dependent on what cues they use and to what extent these are generalised, is therefore an important consideration in designing CFA trials. Crows have high cognitive ability (Emery 2004), and therefore every effort must be made to test the transferability of aversive conditioning to natural nests.

Whether results from artificial nest studies should be used as an accurate representation of natural nest predation is the subject of much debate (Burke et al. 2004; Faaborg 2004; Moore & Robinson 2004). Studies often show that predation rates on artificial nests are higher than for natural nests and variable patterns of predation are not adequately represented by predation rates for artificial nests (Major & Kendal 1996; Burke et al. 2004; Ekanayake et al. 2015). This is often due to concealment or active nest defence by parental birds (King et al. 1996; Burke et al. 2004; Opermanis 2004). Consequently, it is questionable if artificial nest experiments accurately predict species breeding success (Faaborg 2004). Nevertheless, use of artificial nests was appropriate in this study because I undertook a specific balanced experimental manipulation to test a change in nest predator behaviour (Villard & Pärt 2004). In addition I tried to accurately match nest cues for the Kittlitz's Plover by matching nest design and partially burying eggs, a common state for natural nests. Results from a brief pilot study confirm that buried eggs are more likely to survive than exposed eggs (Appendix B), which may partially explain why predation was higher on artificial nests containing partially exposed eggs, than on natural nests which contain eggs that were sometimes covered with nest material. However, in the absence of parental birds as cues to a nest location, it was necessary to partially expose eggs which may otherwise have remained largely undiscovered (Appendix B).

Accurate estimations of nest survival rates are important for bird conservation (Jehle et al. 2004). In this study, the fact that Kittlitz's Plovers are known to remove eggshell remains from the nest soon after the eggs have hatched (Hockey et al. 2005), means there is a chance that

hatched nests may have been mistaken for predated nests. However, the likelihood is small considering that for 2/3 nests that hatched, the brooding adult and chick(s) were found within the nest vicinity and in the case of the other nest the camera trap footage confirmed the hatching event. This was not the case at 7 of the 'predated' nests discovered during the 'laying' period (see methods) and considering that these were found empty within a maximum of 13 days post discovery (average 7, range 2.5 to 13 days) there is no chance that these nests could have hatched (average incubation phase 22-28 days (Hockey et al. 2005)). Therefore there was a reasonable degree of accuracy (10/19 nests) when determining nest fate in the field and therefore in the natural nest survival rates calculated from these observations.

### ***Nest predators and induction of CFA***

The Pied Crow was the only species observed predated both artificial and natural plover nests, either through direct observation or camera trap footage. In agreement with recent findings from the same study site (Troschianko et al. in prep), this indicates that the Pied Crow is the main predator of Kittlitz's Plover nests and probably other ground-nesting species in the area too. Given that corvids have greater negative effects on prey productivity (nest success, brood size) than abundance (Madden et al. 2015), the suspected increase in abundance of Pied Crows (Cunningham et al. 2015) and associated predation pressure as demonstrated here, could have detrimental effects on wader population trends in the area. In particular, the near-threatened Chestnut-banded Plover also suffer nest predation by Pied Crow (Troschianko et al. in prep) and may be an appropriate species to target for protection through induction of CFA. Although nest failure is an important demographic parameter for waders (Macdonald & Bolton 2008), wader population trends are also affected by others factors, such as chick and adult survival, immigration and emigration (Macdonald & Bolton 2008), parameters that were not assessed in this study. Nevertheless our findings and those by Troschianko et al. (in prep) provide some information on the effect of nest predation on Kittlitz's Plover hatching success which could contribute to a more in depth study in the future. The primary aim of this study was to test CFA as a potential tool for use by conservation practitioners to address these high rates of nest predation by Pied Crow should the need arise.

Results of this study provide indirect evidence that CFA was induced in Pied Crows. Crow sightings were less common after treatment with carbachol than before, suggesting that individuals largely abandoned sites following treatment with carbachol. Such site abandonment by crows is common in CFA trials (Dimmick & Nicolaus 1990; Avery & Decker 1994). For example wild American Crows (*Corvus brachyrhynchos*) abandoned sites containing eggs that looked the same as those they had previously been conditioned to avoid and also tended to avoid similar looking eggs even in other areas (Dimmick & Nicolaus 1990). The ability to cause local site abandonment is probably the most promising use of CFA as a tool to protect threatened species. It suggests that even if predators are able to distinguish between artificial and natural nests, predation risk could be dramatically reduced if treated eggs are deployed near important breeding sites. This approach has already been suggested as a way to protect California Least Tern colonies from predation from Common Ravens (Avery et al. 1995). Both the Kittlitz's Plover and the near-threatened Chestnut-banded Plover are localized breeders (Hockey et al. 2005) and known to breed on the commercial saltpans in the Berg River Estuary (Barnes 1998). Therefore these sites could be effectively targeted for deployment of carbachol-treated eggs to deter predators during breeding season.

Indirect measures of CFA in predators were used in this study because of the difficulty in demonstrating that CFA has or has not been induced in individual predators in a short field study. One would need to make repeated observations of individuals consuming treated eggs, becoming ill, and subsequently avoiding eggs that look the same. Such assessments are possible in laboratory studies, but very difficult in field studies, and the responses in these different environments are not necessarily congruent (see Introduction CFA laboratory trials, Gustavson & Gustavson 1985; Nicolaus et al. 1989). Any individual level assessment of illness development, behavioural changes or nest avoidance, would have required crow capture and bird ringing, which in consideration of the numerous logistical difficulties of crow capture (Caffrey 2002) could not be done in this study due to time limitations. In any event, monitoring individuals would have been difficult, as crows often remove eggs and eat them in a different location which may be out of sight (Catry & Granadeiro 2006, A. Ferguson, pers.obs).

Although direct observation of individual predator behavior may be difficult, there may be potential for the use of nest cameras especially those with a video feature to provide information about predator behaviour. Camera trap footage from an artificial nest at a carbachol-treated site six days into the treatment phase revealed a crow landing near the nest, looking down at the eggs and flying away, leaving the nest intact. It was not until six days later that the nest was predated. Although it is possible that this was just a chance event, such an observation may indicate some sort of avoidance or cautionary behavior being displayed by predators as a result of carbachol treatment. Further testing on the use of camera traps for this purpose would certainly be useful as a means to improve our understanding of the response of wild predators to CFA treatment.

### ***Improvements to CFA experimental design***

My results demonstrate that CFA can be induced, and with refinement of the experimental approach the efficacy of CFA could be improved further. In terms of the treatment itself, a number of parameters may be adjusted to optimize efficacy and minimize costs including: (i) exposure time to emetic eggs, egg quantity and density, (ii) emetic chemical and dosage (iii) artificial nest type, and appearance. To a great extent, decisions on these parameters must be based on the predator species identified, their abundance, and their behaviour.

Results of this study echo those of other studies that exposure time to emetic eggs is an important determinant of the magnitude and latency of the effect (Maguire et al. 2009). As time progressed during the treatment phase, the likelihood of nest survival in carbachol-treated plots gradually increased. This is a common result in successful CFA trials, whereby the percentage of eggs or nests predated per unit time gradually decreases as time progresses during conditioning (Nicolaus et al. 1989; Conover 1990), demonstrating that the treatment effect is cumulative as more individuals consume treated eggs. Although a direct analysis of the likelihood of survival during the post-treatment phase was not conducted, the rate of egg loss did appear to be slower at carbachol sites than at control sites. Together these findings suggest that the carbachol effect may be enhanced by prolonging the treatment phase. Other authors have emphasized that an extended period of conditioning is probably necessary in

unpredictable field conditions (Maguire et al. 2009) and many recommend that treatment should last at least as long as the average incubation period of the prey in question (Bogliani & Bellinato 1998). Unfortunately, due to time constraints in this study, it was not possible for the experimental phases to last as long as 22 to 28 days, the average incubation period of the Kittlitz's Plover (Hockey et al. 2005). This is recommended for future studies to enhance the carbachol effect and increase the chances of protecting natural nests.

In addition to inadequate exposure time to treated eggs, the actual dosage consumed would affect the acquisition of CFA. In the absence of a prior laboratory test and considering that carbachol can be toxic if consumed in excess (Conover 1990; Cox et al. 2004), I opted to use a very conservative dosage found to be effective in Steller's Jays (Gabriel & Golightly 2014). However, without behavioural observations at the individual level, one cannot be sure that 31.34 mg carbachol per quail egg is an optimally effective dosage for wild Pied Crows as well. Such an optimal effective dosage would be one that induces a temporary illness rapidly enough for an individual to make the association with the food, as well as severe enough to induce avoidance (Nicolaus et al. 1983; Gill et al. 2000) without long-term damage to any individual predator or other non-target species (Conover 1990; Gill et al. 2000). Dosage determination is important because dosages that are too low do not induce complete or long-lasting avoidance nor do they prevent re-sampling of eggs (Avery & Decker 1994). It is also possible that wild Pied Crows may have consumed only part of the egg contents, and thus did not receive the full dosage. Bogliani & Bellinato (1998) used an elevated dosage of 60 mg carbachol per chicken egg after noticing that wild Hooded Crows (mean body mass 510 g; Robinson 2015), similar in size to the Pied Crow (mean body mass = 520g; Hockey et al 2005), only partially ate eggs containing diluted egg contents. Whether the dosage itself was too low or predators only partially ate the eggs, this would have induced only mild illness, perhaps too mild to elicit long-lasting avoidance or prevent egg re-sampling (Avery & Decker 1994; Bogliani & Bellinato 1998).

Laboratory tests are recommended to determine a dosage suited to the Pied Crow. Ideally this dosage should rapidly induce illness such that individuals are likely to eat only one or a few eggs before avoiding them completely (Nicolaus et al. 1983; Gill et al. 2000) as this will allow for



more eggs to be available for other individuals. These laboratory tests should be coupled with field trials to investigate how many eggs individual wild crows are likely to find, as well as how many eggs and how much of the contents constitutes an average meal (Avery et al. 1995). For practical and ethical reasons, these must be examined as they determine the amount of chemical actually ingested and therefore the degree of aversion induced (Avery et al. 1995) as well as the risks of poisoning (Conover 1990).

Alternative chemicals should also be considered for inducing CFA (Catry & Granadeiro 2006). Although carbachol is probably the most effective chemical for use as aversions can potentially be retained for up to 12 months (Gabriel & Golightly 2011), this chemical is expensive in South Africa (R5436.38 ~ \$342.59 per 10 g), and therefore other substances may be more feasible for practical management use. Laboratory trials should incorporate alternatives which could be equally effective at inducing CFA but more cost effective. Chemicals that are worth consideration are methiocarb and topical methyl anthranilate found to be effective for inducing CFA in captive fish crows (*Corvus ossifragus*) (Avery & Decker 1994), landrin used successfully to reduce nest predation in wild American Crows (Dimmick & Nicolaus 1990), lithium chloride used to induce conditioned taste aversion (CTA) in captive red-winged black birds (*Agelaius phoeniceus*) (Rogers Jr 1974), sucrose octacetate which is a non-toxic bitter-tasting compound that can elicit taste avoidance in red-winged blackbirds (Rogers Jr 1974), as well as cinnamamide, thiabendazole, levamisole, fluoxetine and clotrimazole which all induced strong last-lasting CTA in rats (Gill et al. 2000). These substances should be tested in different combinations, at different dosages and with alternative food available to better simulate a natural environment and because food deprivation could cause fatalities (Rogers Jr 1974). Although the price and availability of most of these chemicals remains to be determined, I found that lithium chloride (R1074.91~ \$67.9 per 500 g, Associated Chemical Enterprises) was considerably cheaper than carbachol and therefore perhaps a more feasible option for conservation management.

To maximize effectiveness and increase the likelihood of benefits afforded to natural nests, carbachol-treated eggs should be provisioned long in advance of the start of the breeding

season (Conover 1990; Avery & Decker 1994; Bogliani & Bellinato 1998; Maguire et al. 2009) and continue throughout the breeding season (Baker et al. 2005). Not only is this likely to enhance the carbachol effect through an extended exposure time, but it would also shorten the timeframe in which crows might sample plover eggs and therefore learn to distinguish 'safe' plover eggs from treated quail eggs (Cox et al. 2004; Baker et al. 2005). Additionally, aversions take time to develop, known as a 'learning curve' (Baker et al. 2005), therefore the sooner crows are exposed to treated eggs the sooner they can learn to avoid them.

Whilst there was a strong effect of carbachol to increase artificial nest survival after treatment, it is still important to note that few nests survived to the end of the post-treatment phase. A possible explanation for this is that some individuals were not exposed to treated eggs during the treatment phase. Crows were noticeably abundant in the study area, therefore it is possible that there were simply too many crows and not enough treated eggs to feed them all. Dimmick & Nicolaus (1990) show that CFA failed to develop in crows when the number of baits was insufficient for all the crows. On the other hand the provision of too many eggs can have the opposite effect and actually attract predators to a seemingly productive foraging area or make them more reluctant to completely abandon the site (Martin 1988; Dimmick & Nicolaus 1990). Either of these scenarios could have occurred and would explain why predation continued into the post-treatment phase when crows may have learnt that eggs were once again safe to eat. In a future trial, the abundance of nests should be varied according to predator density and sampling frequency prior to the conditioning phase to determine which density of treated nests is most effective for inducing CFA (Dimmick & Nicolaus 1990).

Certainly more information is required about the abundance of predators in the area, particularly crows, as well as their spatial distribution and movement patterns. These factors influence how many treated eggs should be deployed, at what density and for how long (Nicolaus et al. 1989). Possible immigration of unexposed individuals, perhaps even some individuals replacing those that abandoned the site (Bogliani & Bellinato 1998), could explain why nest predation continued during the post-treatment phase. In addition to extending the treatment phase, one might consider regular replacement of eggs or repeat deployments to

maximise exposure and ensure that any new or transient individuals are also exposed to treatment (Avery et al. 1995; Bogliani & Bellinato 1998).

Identification of the Pied Crow as the chief nest predator has major implications for research in the area as well as for the development of CFA as a tool to manage plover nest predation. Given that Pied Crows are diurnal predators that mainly forage on the ground, it is likely that they use several visual cues to detect nests (see Appendix B). There is more work to be done in this regard, as little is known about the foraging tactics of Pied Crows, and in particular what tactics they use to target wader nests. Such information is required such that the cues available at artificial nests can be closely matched to natural cues used by Pied Crow.

Results of this study demonstrate that CFA is a promising tool for conservation management. However, before CFA is implemented as a means for controlling Pied Crow and other predators further research needs to be done: 1) the impacts of Pied Crows, particularly on already threatened species, need to be thoroughly investigated, documented and evaluated relative to other threats, 2) the CFA experimental design requires development, particularly with respect to assessing how treatment could affect natural nests.

### ***Conclusions***

This was the first field trial to demonstrate the potential use of Conditioned Food Aversion (CFA) as a conservation management tool to reduce nest predation in Africa. The experimental design used here is an advance on previous studies as it makes use of both spatial and temporal controls and incorporates quantification of predator identity and abundance such that the effects on predation can be attributed to carbachol treatment with greater certainty. The results show that the Pied Crow can be added to the list of corvid predators that can learn to avoid artificial plover nests using treatment with carbachol. More specifically, the deployment of carbachol-treated quail eggs can elicit at least partial site abandonment resulting in increased survival rates of artificial plover nests, and perhaps natural nests too, although this later benefit remains to be confirmed. As the main identified nest predator, the need to investigate the ecological effects of increasing Pied Crow abundance in certain regions of South

Africa is re-iterated. Resources for conservation are limited necessitating prior testing of potential management tools to assess whether objectives can be met (Madden et al. 2015). Refinement of the CFA technique is possible and should be conducted to increase the efficacy of treatment and maximise the possibility that natural nest survival can be increased for species threatened by nest predation. With a modified technique, there is every chance that CFA could become a valuable and practical conservation tool to protect threatened plovers in the region, such as the near threatened Chestnut-banded Plover.

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**Appendix A: Comparison of Kittlitz's Plover eggs with Japanese quail eggs**



Figure A1. A Kittlitz's Plover (*Charadrius pecuarius*) egg (middle) from an abandoned nest in comparison to four Japanese quail (*Coturnix japonica*) eggs of various sizes, shape and pattern.



## **Appendix B: Pilot study to investigate effect of egg-covering on nest predation**

### ***Rationale***

The cues used by predators to locate nests, as well as the anti-predator tactics employed by incubating birds are strong determinants of nest predation (King et al. 1996; Burke et al. 2004; Opermanis 2004; Šálek & Zámečník 2014; Ekanayake et al. 2015; Ibáñez-Álamo et al. 2015). Kittlitz's Plovers are known to kick sand and other nest material over their eggs (Fig. B1b), especially when disturbed (Johnsgard 1981; Hockey et al. 2005), suggesting that this is an anti-predator tactic employed by this species to protect their eggs. To test this, I ran a brief one week pilot study (18<sup>th</sup> to 26<sup>th</sup> November 2015) to answer two separate but associated questions: 1) what tactics do predators use in nest predation and 2) what tactics do breeding birds employ to reduce nest predation? Such questions are important to consider when designing CFA experiments because they will affect the capacity for predators to learn appropriate cues to emetic eggs. To answer these questions, I conducted a paired experiment to compare the survival of 'buried' nests with 'exposed' nests.

### ***Experimental design***

Three areas at least 2 km apart were chosen in suitable Kittlitz's Plover nesting habitat and such that they were at least 500 m away from any CFA plot to prevent interference with the main CFA experiment. The pilot study took place late into the post-treatment phase of the main CFA experiment and only overlapped by 4 – 7 days (2 – 4 checks) with nearby CFA sites (C, D and E). Furthermore, by the start of the pilot study all but one of the artificial nests had already been predated at those sites. Therefore I was reasonably certain that any interference with the main experiment was minimal and inconsequential.

Eight nest pairs were created at 100 m intervals along each of three roughly linear transects (1 transect per area). Nests in a pair were created 5 m apart but were otherwise created similarly to those in CFA plots in shallow scrapes containing quail eggs. In this experiment only 'exposed' nests contained eggs that were 50% buried (Fig. B1c) whereas 'buried' nests had eggs that were completely covered with up to 2 mm of soil (Fig. B1d). Nests were checked for damage once

every day at approximately the same time (morning, midday or evening respectively) and all pairs in a transect were checked in one session. Whether nests were predated or survived was defined as for the main CFA experiment (see Methods, Artificial nests, (ii) Data collection).

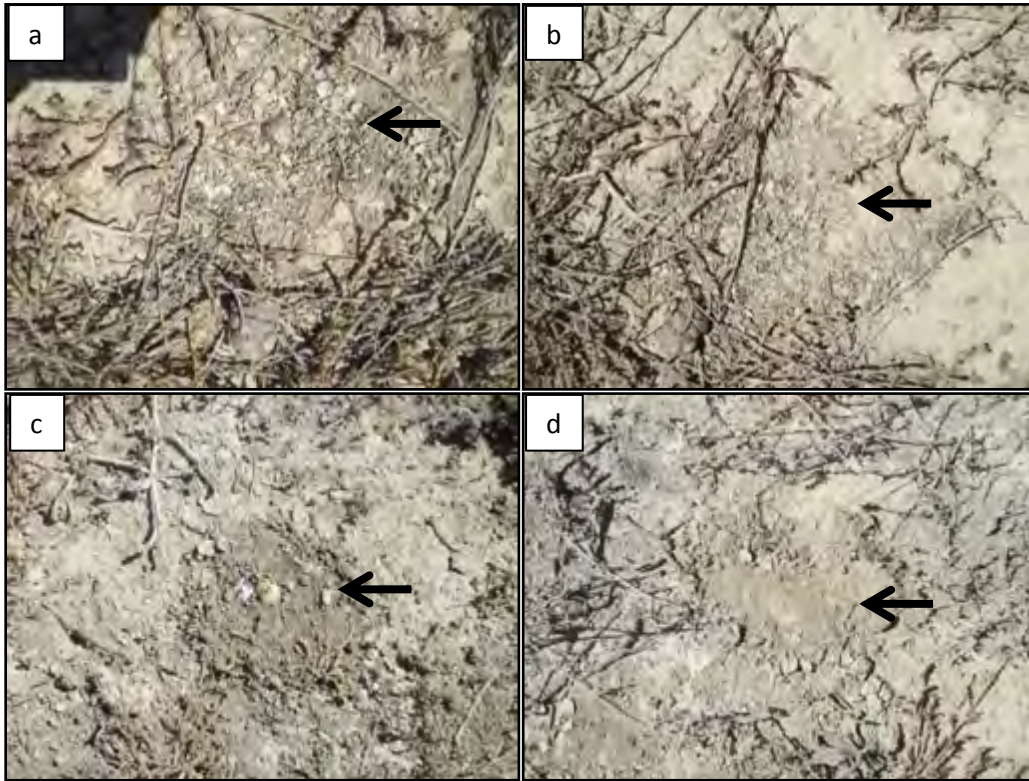


Figure B1. A Kittlitz's Plover (*Charadrius pecuarius*) nest a) with eggs partially exposed and b) with eggs completely covered with nest material kicked up by the departing adult after disturbance, compared with c) an artificial nest with Japanese quail (*Coturnix japonica*) eggs partially exposed and d) its paired nest (5 m away) with eggs completely buried.

### **Data analysis**

Paired nest data from this experiment was analysed using a GLMM with the negative binomial distribution to account for over-dispersion, using the R package 'glmmADMB' (Skaug et al. 2015). The response variable was number of checks survived (1 check = 1 day), and level of egg cover (buried / exposed) was the explanatory factor considered. Transect was added as a random factor with nest pair code nested within this.

The final model took the form:

```
glmmadmb(checks.survived~egg.cover+(1|transect/pair),family="nbinom",
          zeroInflation = FALSE)
```

### Results

All 24 exposed nests were lost by the end of the 7 day experiment, whereas only 5 out of 24 buried nests were lost. The negative binomial GLMM for the paired nest data showed that egg cover had a significant effect on the number of checks survived by nests in a pair ( $Z = 1.16$ ,  $p < 0.001$ , Table B1). Buried nests survived more than three times as many checks as exposed nests (buried: 6.2 [+1.80: -1.39], exposed: 1.9 [+0.49: -0.39], Fig. B2).

Table B1. Summary results of the negative binomial GLMM showing the effect of egg cover (after accounting for the effect of transect and nest pair) on the number of nest checks survived by artificial nests created in the Berg River Estuary ( $n = 3$  transects, 8 buried nests and 8 exposed nests per transect).

Model term	Level	Effect $\pm$ S.E.	Z	P
Intercept		0.66 $\pm$ 0.226	2.93	<b>0.003</b>
Egg cover	Buried	1.16 $\pm$ 0.184	6.3	<b>&lt; 0.001</b>

Significant  $p$  values are in **bold**

† Exposed was the reference category

†† Transect was a random effect in the model, with nest pair code nested within this

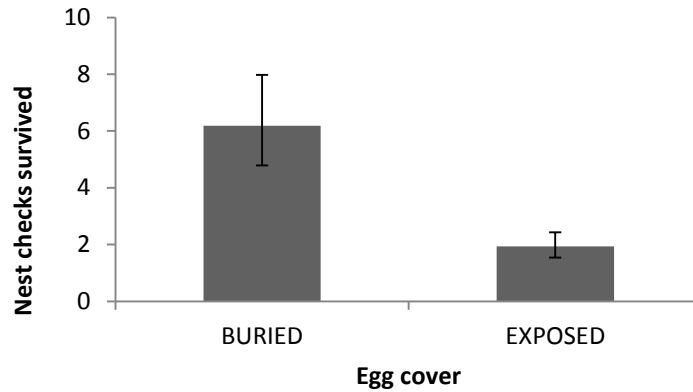


Figure B2. Negative binomial GLMM showing that artificial nests containing buried eggs survived significantly more checks than nests where eggs were exposed. (Values are given as a back-transformed mean  $\pm$  1 SE,  $n = 48$  nests (3 transects, 8 buried nests and 8 exposed nests per transect)).

### Discussion

The results of this pilot study show that buried nests survived considerably longer than exposed nests (three times longer). This provides strong support for egg burying as an effective anti-predator strategy and suggests that the main predators of plover nests in the Berg River Estuary use visual cues, in this case eggs, to locate artificial nests. Although predation of natural nests may principally occur where eggs remain unburied, it is likely that predators additionally use the presence of an incubating parent, or parental behaviour as a cue to locate buried eggs. Other studies have revealed similar results, for example Opermanis (2004) used artificial ground-nests as models for mallard duck (*Anas platyrhynchos*) and northern shoveler (*Anas clypeata*) nests and demonstrated that nests covered with down were found significantly less frequently by marsh harriers (*Circus aeruginosus*), the main nest predator, than nests with visible eggs. Similarly, marsh harriers were thought to use parental bird movements as a cue to locate natural nests.

Direct observations of adult Kittlitz's Plovers at natural nests ( $n = 19$  nests) during the entire study period (25<sup>th</sup> September to the 27<sup>th</sup> November 2015) indicated that there is variation not only in the degree of egg burying, but also in the likelihood that the eggs are buried at all. In the

majority of cases, an incubating adult was seen kicking sand over the eggs when disturbed off the nest. However, in some instances, as an observer approached the nest, an adult bird was seen returning to the nest from some distance away in order to cover the eggs. This provides anecdotal evidence that incubating Kittlitz's Plovers display egg covering behavior as an anti-predator response but may leave eggs exposed during an incubation recess or during incubation change over, probably to prevent overheating (Amat et al. 2012). This is in agreement with published information about the species (Johnsgard 1981) and suggests that egg-covering is an anti-predation strategy rather than for nest thermoregulation. However, given that egg covering for thermoregulatory purposes is also dependent on time of day and ambient temperatures, neither of which were assessed in this study, it is possible that it has a dual function in the Kittlitz's Plover, as demonstrated for Kentish Plovers (*Charadrius alexandrines*) (Amat et al. 2012).

These observations and results from this pilot study also justify the decision to leave quail eggs 50% exposed in plots with artificial nests for the CFA experiment. In the absence of parent bird behaviour, this level of cover reflected a possible natural scenario while also allowing for treated eggs to be detected and consumed by foraging predators, a requirement for CFA to be induced. Consequently this maintained comparability between natural and artificial nests.

## Appendix C: Camera trap images of predators at artificial plover nests

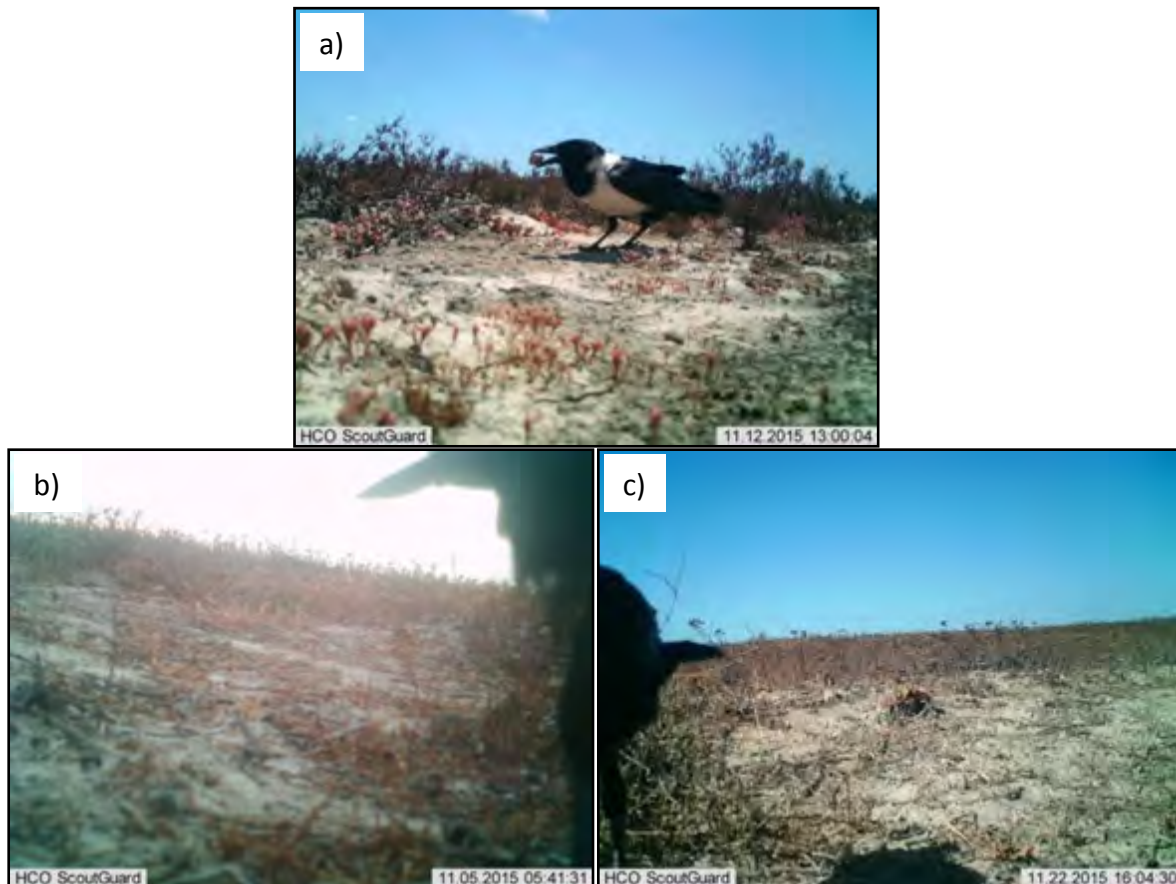


Figure C1. Photographs taken by motion sensitive trail cameras set up at 3 different artificial plover nests during a CFA trial near the Berg River Estuary, South Africa. In a) the predator is 'unambiguously' identifiable as a Pied Crow (*Corvus albus*). In photographs b) and c) the potential predator is 'ambiguous' but assumed to be a Pied Crow based on the black feathers (b and c), short black lower mandible (b) and black legs (c).