

The effect of increased perceived predation risk on parental investment in dark-capped bulbuls (*Pycnonotus tricolor*)



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## Abstract

Parental investment plays a crucial role in life-history traits of birds; and it is highly dependent on the energetic costs of raising the current brood, compared to the relative success. Hence, how much energy a parent should invest in their current offspring, to maximise its fitness, is often influenced by the chance of nest predation as well as future opportunities.

Temperate birds are known to respond to increased predation risk by investing less in eggs (e.g. smaller and fewer eggs), whilst decreasing their time near the nest and increasing the duration of incubation bouts to minimise the costs of a possible predation event. In sub-tropical habitats, however, predation risk is substantially higher than in temperate regions, resulting in generally small clutches with small eggs. This raises the question of whether such responses can be ubiquitous across different climates. We aimed to test if passerines that inhabit subtropical savannas, and that evolved under high predation pressure, exhibit similar responses to increased perceived predation risk as temperate species. We studied the dark-capped bulbul (*Pycnonoctus tricolor*), a medium-sized southern African passerine that breeds in a socially monogamous biparental care system. To determine the effect of increased perceived predation risk on parental investment, we played predator call playbacks on the day the first egg was laid. We then examined the effect on subsequent investment in clutch- and egg size, nest attentiveness and incubation duration. Dark-capped bulbuls did not appear to alter any of the measured forms of investment with increasing perceived predation risk. We concluded that dark-capped bulbuls did not exhibit similar plastic responses to predation risk as temperate passerines. Perhaps plasticity in clutch- and egg size is limited due to naturally small clutch- and egg sizes in sub-tropical birds. This illustrates that parental investment strategies vary across climates and are contingent on how much such strategies were already optimised as a response to natural circumstances.

Keywords: birds; savanna; southern Africa; parental investment; *Pycnonoctus tricolor*; predation risk.

## Introduction

Life history traits in birds are one of the most studied topics in evolutionary ecology (Lima, 2009). To maximise their fitness, parents have to make decisions about how much energy to invest in either survival or reproduction. The energy spent on current reproduction cannot be invested in future reproduction or parental survival. Hence, with natural selection working on the fitness of the parents, creating trade-offs, it forces them to optimise their strategy (Badyaev & Ghalambor, 2001; Slagsvold, 1984; Smith et al., 1989). These life-history decisions are however highly influenced by current environmental conditions. Researchers have attempted to determine what environmental factors birds base their decision on, and which internal mechanisms are affected by them. Food limitation (Ferretti et al., 2005; Martin & Briskie, 2009; Smith et al., 1989) and thermoregulation (Nager & Zandt, 1994; Ojanen et al., 1981) were long thought to be the main drivers of reproductive decisions made by parents because they influence the current energy levels and thus long-term survival of the females. When multiple studies revealed that this was not the case, predation became the focal point in following studies (Creel & Christianson, 2008; Ferretti et al., 2005; Fontaine & Martin, 2006; Lima, 2009; Lloyd, 2004; Preisser et al., 2005). Research then found that not only predation of the eggs and nestlings had a great effect on reproductive trade-offs, but also the vicinity of predators is believed to play a crucial role (Lima, 2009; Martin & Briskie, 2009; Preisser et al., 2005; Sarkar & Khajanchi, 2020; Travers et al., 2010; Zanette et al., 2006; Zanette et al., 2011). Therefore, investigating the effect of not only predation but also predation risk on parental investment is crucial to gain a better understanding of the mechanisms that drive life-history decisions in birds and how they are affected by the environment.

Predation is known to have a great shaping effect on the behaviour, life-history traits and decisions of animals, as it plays a key role during the life of prey species (Lima, 2009; Martin & Briskie, 2009; Sarkar & Khajanchi, 2020). In birds, nest predation is the cause of 70%-90% of all reproductive failures (Martin & Briskie, 2009). Therefore, it was long believed that demographic changes in prey populations were exclusively due to direct killing (Zanette et al., 2011). However, it has become clear over the years that perceived predation risk in itself is powerful enough to shape prey demography (Preisser et al., 2005; Sarkar & Khajanchi, 2020; Travers et al., 2010; Zanette et al., 2006; Zanette et al., 2011). Predation risk fluctuates throughout the life of prey species. Therefore, it is important for individuals to assess and adjust to changes in predation risk in terms of behaviour and reproductive strategies (Lima, 2009). Rapidly changing and unpredictable environments ask for greater adaptability, resulting in phenotypic and behavioural plasticity in many important traits (Lima, 2009; Martin & Briskie, 2009).

To minimise the risk of nest predation, parents often primarily resort to behavioural changes (Conway & Martin, 2000; Ghalambor & Martin, 2002; Ghalambor et al., 2013; Lima, 2009; Martin et al., 2000; Morosinotto et al., 2013). For example, increased vigilance will provide crucial information about the current risk of their nest getting predated. When the risk is too high, and the current investment is limited, birds tend to relocate their nesting site (Lima, 2009; Martin & Briskie, 2009; Zanette et al., 2011). However, if predation pressure increases when the parents have already invested considerably in the current nesting site, they adjust their parental behaviours to minimise the risk of predation (Arnold, 1991; Badyaev & Ghalambor, 2001; Eggers et al., 2006; Ghalambor et al., 2013; Lima, 2009; Martin & Briskie, 2009; Slagsvold, 1982, 1984; Slagsvold et al., 1984; Smith et al., 1989; Walker et al., 2008). For example, nest attentiveness was found to increase with an increase in predation risk in several Northern American passerines (Conway & Martin, 2000) such as the brown creeper (*Certhia americana*) (Ghalambor & Martin, 2002), and in great tits (*Parus major*) (Basso & Richner, 2015). By staying on the nest, females are able to defend the eggs and make sure the nest concealment remains high. To do so, females rely on their camouflage and try to blend into their

surroundings, thus keeping the nest hidden (Lima, 2009; Martin & Briskie, 2009). Similarly, with an increased predation risk during incubation, females of the rufous-bellied thrush (*Turdus rufiventris*) (Ferretti et al., 2005), the small ground finch (*Geospiza fuliginosa*) (Kleindorfer, 2007) and the yellow-eyed junco (*Junco phaeonotus*) (Weathers & Sullivan, 1989) increased the duration of their incubation bouts, thereby minimizing the number of visits to the nest (Lima, 2009; Martin & Briskie, 2009). Parents are also known to decrease their provisioning behaviour to maintain crypsis of the nest (Martin & Briskie, 2009), which impacts the nestling growth rate (Creel & Christianson, 2008). Thus, by adapting their behaviour, parents are able to minimise the risks and costs of predation on their current reproductive attempt.

When minimising the risk of nest predation itself is not possible, birds have to use other strategies to minimise the risk, or the cost, of losing their current offspring. Multiple studies have reported physiologically plastic changes in clutch size, egg size, sex ratio, egg quality and hatching pattern due to increased predation risk (Arnold, 1991; Badyaev & Ghalambor, 2001; Eggers et al., 2006; Ghalambor et al., 2013; Lima, 2009; Martin & Briskie, 2009; Slagsvold, 1982, 1984; Slagsvold et al., 1984; Smith et al., 1989; Walker et al., 2008). As such, according to the nest predation hypothesis, parents should reduce their reproductive efforts when nest predation is high by for example laying smaller clutches (Eggers et al., 2006; Lima, 2009; Martin & Briskie, 2009; Slagsvold, 1982, 1984; Slagsvold et al., 1984; Travers et al., 2010; Zanette et al., 2011). Zanette et al. (2011) indeed found that under heightened perceived predation risk, song sparrows (*Melospiza melodia*) decreased their clutch size and increased their egg size. Smaller clutches take less time to complete, enabling the female to expedite the onset of incubation. The earlier the chicks hatch and thus fledge, the less time the brood is at risk (Eggers et al., 2006; Martin & Briskie, 2009; Perrins, 1977; Slagsvold, 1982, 1984). Moreover, such a reduction in clutch size, when predation increases, reduces the costs of losing the current brood and is perhaps the result of a larger focus on future reproduction in a trade-off. However, Fontaine & Martin (2006) and Martin & Briskie (2009) found the opposite in several different temperate passerines (e.g. the house wren (*Troglodytes aedon*), the Cordilleran flycatcher (*Empidonax occidentalis*), the hermit thrush (*Catharus guttatus*), the American robin (*Turdus migratorius*) and the grey-headed junco (*Junco hyemalis*)), illustrating that the effects of predation pressure on clutch- and egg size may be species-specific.

Predators often don't only predate on the eggs or nestlings. They may, in some cases, also pose a threat to the adults. In addition to nest predation, birds also have to take the predation risk to themselves into account when deciding how much energy to invest in their current reproductive attempt. In situations where parental survival, as well as brood survival, are at stake, it's of utmost importance that the parents carefully consider what decision is going to give them the maximal fitness: risking their survival for their current brood or risking the brood survival for their own (Badyaev & Ghalambor, 2001).

In more tropical regions, such as the southern African savannas, ambient predation risk is substantially higher than in temperate regions (Martin, 1996; Martin et al., 2006). Evolving under such high predation pressure has resulted in sub-tropical species laying exceptionally small clutches with small eggs in southern Africa (Martin, 1996; Martin et al., 2006). As evident from the previously described theoretical framework, our knowledge of how temperate species alter their parental investment, to limit the costs of predation on current offspring, has been well-researched. Yet, our knowledge on the main drivers of these life-history decisions, and plasticity in parental investment in savanna passerines is lacking. This is surprising given the prominent role of tropical species in studying life-history evolution. Knowing the vast difference in environment that shaped their life-history traits, we cannot simply imply that temperate passerines and southern African passerines adopt similar strategies. Perhaps sub-tropical passerines cannot decrease their investment much further without jeopardising healthy offspring. Thus, to advance our fundamental knowledge of how parental

investment is affected by predation risk, and what affects life-history decisions in savanna passerines, we need to commence research on this topic.

To our knowledge, we are the first to examine if there is an effect of increased perceived predation risk on the parental investment of southern African passerines, using the dark-capped bulbul (*Pycnonotus tricolor*) as model species. The dark-capped bulbul as a species is ideal for this framework because they are abundant in the study area, provide biparental care to altricial offspring and nest in open cup nests; making them especially susceptible to nest predation (Eggers et al., 2006; Liversidge, 1970; Martin & Briskie, 2009; Perrins, 1977). Parental care consists of many aspects, and in order to know which of these aspects are more affected by predation risk, we investigated specifically the changes in clutch size, egg size, nest attentiveness and incubation behaviour under increased perceived predation risk, whilst controlling for ambient predation risk. We hypothesise that increased perceived predation will affect parental investment in bulbuls similar to what has been observed in temperate passerines. We used an experimental within-brood design, which enabled us to control for most environmental factors, including the natural habitat, predation risk and predator density. Therefore, we could isolate the effects of an increase in perceived predation risk, which allowed us to ascribe the found effects to the role perceived predation risk plays on parental investment.

With this study, we aimed to investigate whether sub-tropical passerines exhibit similar phenotypic and behavioural plasticity to increased perceived predation risk, as passerine birds in temperate climates (e.g. song sparrow (Zanette et al., 2011), great tit (Basso & Richner, 2015), Siberian jay (*Perisoreus infaustus*) (Eggers et al., 2006), etc.). We tested plasticity by playing predator call playbacks in the proximity of the nest, to increase perceived predation whilst maintaining the actual predation risk. Additionally, we controlled for natural predation risk by using the number of seen and heard predators around each nest as a proxy for ambient predation risk. We predict that increased perceived predation risk results in an increase in energy expenditure in nest defence, but a decrease in energy expenditure in egg production. This means that with increased perceived predation risk, we expect to find smaller clutches, with smaller eggs, as well as an overall higher percentage of time spent on the nest with limited visits to the nest, for both nest attentiveness during the laying cycle and incubation. Taken together, this study will reveal whether savanna passerines living in a more sub-tropical climate exhibit similar plastic responses in parental investment to predation risk as temperate passerines.

# Methods

## Study site and species

We studied the dark-capped bulbul population in the Mbuluzi Game Reserve, eSwatini (26°09'S, 31°59'E) between mid-September 2022 and mid-January 2023. The four km<sup>2</sup> field site is characterised by savanna and riparian vegetation, with the Mbuluzi River flowing through the landscape.

The dark-capped bulbul is a medium-sized passerine bird native to southern Africa and is one of the three most abundant bird species in eSwatini (Monadjem, 2004; Monadjem, 2005; Moyle & Marks, 2006; Wambura, 2009). They reside in tree-covered habitats ranging from wooden grassland to dense bushveld, where they nest on the edge of the canopy of trees and shrubs (Hockey et al., 2005; Liversidge, 1970; Malan & Lerm, 2013). Bulbul nests are cup-shaped and predominantly built by females. The pair can initiate multiple nest attempts throughout the breeding season (September – mid-February), containing two to three eggs per clutch. Eggs are usually laid at a 24-hour interval, and incubation starts when the clutch is completed. Incubation lasts approximately 12 days, with a slight variation in hatching from 11 up to 14 days (Hockey et al., 2005; Liversidge, 1970; Sinclair et al., 2020; Tarboton, 2011). Nest predation is high (personal observation), and is, therefore, the main limiting factor to the bulbuls' breeding success. The main predators of the dark-capped bulbul in eSwatini include various species of snakes, vervet monkeys (*Chlorocebus pygerythrus*), mongooses, and several bird species (Liversidge, 1970).

## Experimental design

Nests were found through daily nest searches by walking transects in the game reserve, maintaining an approximate distance of 30m between each transect. All active nests were monitored subsequently every four days, during routine visits, to collect data on clutch- and egg size, predation rates and parental investment. All newly found, empty bulbul nests were visited and monitored daily for seven executive days; or until the first egg was laid. When the first egg was found, the experiment was placed. Details about the experimental setup will be provided in the next paragraph. If the nest remained empty after seven days of daily monitoring, the nest was declared inactive and was no longer monitored. The monitoring of the nests was done in the morning, as close to egg-laying as possible, to keep the chance of predation low and to maximise the exposure to the experiment. To study the effects of perceived predation risk on parental investment in dark-capped bulbuls, we played predator call playbacks near the nest during the egg-laying period. The experiment consisted of two treatments (predator playbacks (treatment) and non-predator playbacks (control)) as well as the natural situation (no playbacks). Several parameters considering parental investment and predation risk were measured: clutch- and egg size, nest attentiveness and incubation behaviour (see Table 1 for specifications). We defined nest attentiveness as the presence of one or both parents near or on the nest during the egg-laying stage of the current reproductive attempt. Whereas incubation behaviour was defined as one of the parents, mostly the female, sitting on the nest incubating the eggs.

The experiment was set up when the first egg was found, which served as a within-clutch control for egg size. This intra-clutch control enabled us to ascribe any effects of the playbacks to the experimental treatments, whilst controlling for environmental factors. A camcorder (Sony camcorder HDR-CX405) was placed approximately 3m from the nest to record nest attentiveness. For the playbacks, one speaker (Sonume Go) was placed 20m away from the nest with the speaker facing the nesting site. Songs or calls of all birds used in the playbacks were downloaded from Xenocanto.org (Xeno-canto Foundation); alarm calls were not used. All calls were recorded in eSwatini or surrounding countries and had negligible background noise. Playbacks used for the predator treatment were different calls from: Burchell's coucal (*Centropus burchellii*), African harrier hawk (*Polyboroides typus*), grey-headed bushshrike (*Malaconotus blanchoti*) and pied crow (*Corvus albus*) (Hockey et al., 2005;

Sinclair et al., 2020; Tarboton, 2011). Playbacks used in the non-predator treatment were different calls from: southern black tit (*Melaniparus niger*), black-collared barbet (*Lybius torquatus*), emerald spotted wood dove (*Turtur chalcospilos*) and black-headed oriole (*Oriolus larvatus*) (Hockey et al., 2005; Sinclair et al., 2020; Tarboton, 2011). The playbacks played for two minutes, with a 14-minute interval (Fig. 1). Each species' playback plays once an hour. Recordings were normalised and made into one audio file per treatment in Audacity® version 3.2.1 (Audacity®, 2022; Kingma & Weenink, 2022). The breeding pair was exposed to the playbacks for a minimum of two hours and a maximum of nine hours, for one day. Speakers and camcorders were placed between 05.00h and 09.00h and collected at 17.00h, or earlier when raining. Incubation behaviour was filmed approximately six days post-experiment, following the same protocol. During the recording of the incubation behaviour, no additional playbacks were played. For a detailed fieldwork planning, see Tables 6 and 7 in the appendix.

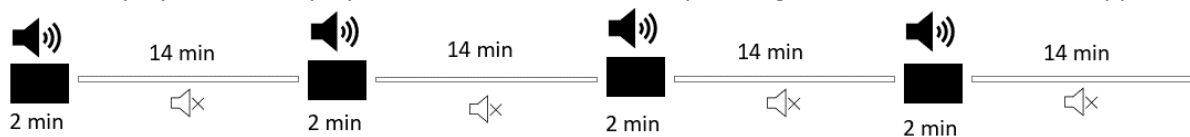


Figure 1: Visual representation of the timing of playbacks played during the experiment. The filled rectangles represent the playing of the playbacks, each filled rectangle represents a playback of a different species, playing for two executive minutes. The empty rectangles represent the 14 minutes of silence between each playback.

## Data analysis

### Parental investment

A total of six variables relating to parental investment were measured and used for further analyses: (1) clutch size, (2) egg volume (mm<sup>3</sup>), (3) proportion of time spent guarding the nest (%), and (4) proportion of the time spent incubating (%), (5-6) number of visits to the nest per hour for both nest attentiveness and incubation. Clutch size was determined by counting the eggs after clutch completion. The egg number was indicated by marking the bottom of the egg with dots corresponding to the laying order (e.g. the first-laid egg of the clutch was marked with one dot, the second egg with two dots respectively). Egg length and breadth were measured for each egg and egg volume was calculated using the following formula:  $V = Kv * LB^2$ , with Kv valued at 0.51, L denoting length and B denoting breadth (Hoyt, 1979). The first and second egg were measured on the day of laying. The third egg, if laid, was measured during the next routine visit. For measuring egg size, an electronic calliper with a precision of one-hundredth of a millimetre was used.

Nest attentiveness and incubation behaviour were recorded as previously described. Videos were analysed using the software program BORIS version 8.11.1 (BORIS, 2023). For the analyses of the videos, we identified the following mutually-exclusive behavioural states for both nest attentiveness and incubation behaviour: (1) the bird was visible near the nest, but not incubating, (2) actively incubating, (3) focal subjects were not visible on camera and (4) other interesting events and behaviours such as predation. We then calculated the amount of time parents spent on each behaviour as a proportion of the observed time. When calculating the number of visits to the nest, we also accounted for the duration of the observed time. For further analyses, only parental care-related behaviours were used (Table 1).

Table 1: Variables related to parental investment and predation risk with their measuring units, used for analyses.

Category	Measured variables	Measuring unit
<b>Parental investment</b>		
Clutch size	Number of eggs in clutch	Count
Egg size	Volume ( $V = Kv * LB^2$ )	Cubic millimetre (mm <sup>3</sup> )
Nest attentiveness	Time spent guarding the nest	Percentage (%)
	Number of visits to the nest	Count
Incubation behaviour	Time spent incubating	Percentage (%)
	Number of incubation bouts	Count
<b>Natural predation risk</b>		
Predator density	Number of predators seen <300m of the nest	Count

### Natural predation risk

To determine the ambient predation risk, predator sightings and hearings were recorded during the daily nest searches. Waypoints for nests and predators were entered in BaseCamp version 4.7.4 (Garmin BaseCamp, 2021). To determine the natural predation risk per nest, a radius of 300m per nest was drawn (Fig. 2). The total number of predators within the 300m radius was counted during the entirety of the fieldwork season. This radius is based on prior studies and literature that determined predator ecology, home ranges and movement (Barrett, 2005; Durner & Gates, 1993; Hockey et al., 2005; Phillips, 1995; Reading, 2012; Roux, 2017; Row & Blouin-Demers, 2006; Sinclair et al., 2020). If parts of the area within the 300m radius were not searched, due to for example the river, we corrected for the area that was searched by looking at the proportion of the radius outside of the search area. We assume that the search area was searched once a week, each week. As an additional proxy for predation risk, we measured the shortest distance from each nest to the Mbuluzi River, using the measuring tool in Google Earth version 9.186.0.0 (Google, 2023), assuming that predation risk is higher closer to the river (Kingma & Bebbington, 2023).

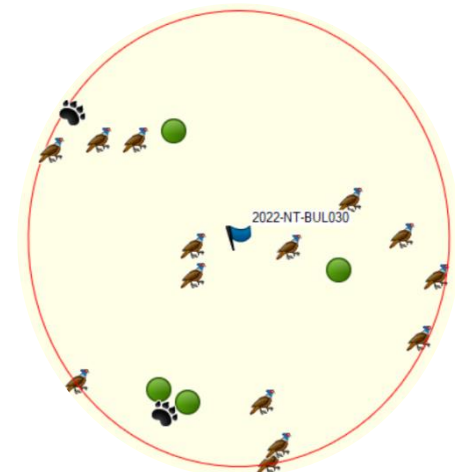


Figure 2: a 300m radius drawn around nest 2022-NT-BUL030 in BaseCamp. The nest is depicted with a flag, predator waypoints are depicted with other symbols. (Sphere = reptilian, pawprint = mammalian, pheasant = avian).

### Statistical analysis

Statistical analyses were performed in RStudio version 4.1.1 (RStudio Team, 2021), with the use of package lme4 version 1.1-32 (Bates et al., 2023). For all tests, statistical significance was inferred if the p-value was < 0.05. The normality of the dependent variables was tested beforehand, using a histogram accompanied by a Shapiro-Wilk test. After testing, post hoc tests between the treatments were done by releveling the treatments. Changing the baseline level of the used factors in the model, allowed us to make comparisons between the different treatments.

We tested for a difference in clutch size between treatments, using a generalized linear model with Poisson error (N= 18 nests), with clutch size as the dependent variable and treatment as the independent variable. We determined clutch size by using the rule of thumb that the clutch had to remain complete (not predated) for two executive nest checks thus, approximately eight days. We used this rule of thumb to ensure that the intention of the parents regarding clutch size was clear. We



thus excluded nests that got predated before potentially completing the clutch. Note that the sample size for this analysis is therefore reduced.

To test for the effect of our treatments (control-predator-natural situation) on egg size, we performed a linear mixed model (N= 59 eggs from 27 nests) with egg volume as the dependent variable. We included treatment, laying order and predator density as fixed factors; and added an interaction term between laying order and treatment. We included nest id as a random factor because eggs from the same nest were laid by the same female and are therefore not independent from each other. After testing, we reduced the model by excluding non-significant factors such as predator density and the interaction term, while retaining treatment as independent variable regardless of whether it was a significant predictor.

To test the nest attentiveness during egg laying, we used two tests. The first test was a general linear model (N= 12 nests) with total number of visits to the nest per hour as the dependent variable and treatment (predator-control) as the independent variable. Number of visits to the nest per hour was calculated as: *total visits to the nest/ total hours observed*. In the second model, a general linear model (N= 12 nests), we tested if there was a relationship between time spent guarding the nest and the treatment. Time spent guarding the nest was calculated as: *((total time spent near the nest + total time spent on the nest)/total time observed)*. For incubation behaviour, we used similar tests as we used for nest attentiveness, with a general linear model (N= 8 nests) for the number of visits to the nest per hour; and a general linear model (N= 8 nests) for the time spent incubating.

## Results

We exposed a total of nine bulbul pairs to an increase in perceived predation risk (predator treatment), and four pairs to playbacks of non-predatory birds (control treatment). From 14 nests, the data was collected outside of the experiment, reflecting the natural situation.

### Clutch size

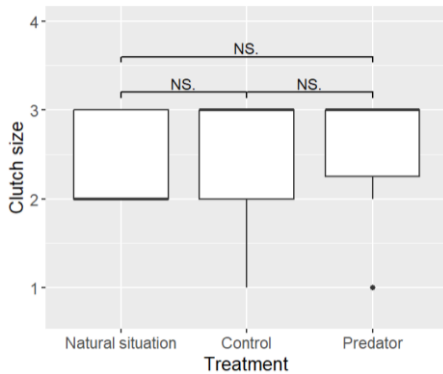


Figure 3: Boxplot showing the difference in clutch size between experimental treatments (control (N=3) – predator (N=6)) and the natural situation (N=9). With treatment depicted on the x-axis and clutch size depicted on the y-axis. Significance indicators indicate non-significant results between all groups.

Experimentally increasing the perceived predation risk did not significantly alter the clutch size of the dark-capped bulbul ( $mean \pm S.E. = 9.163e-01 \pm 4.577e-01$ ,  $p = 0.880$ ). When comparing clutch size of both treatments (control – predator) with the natural situation, no significant difference was found (Table 2; Figure 3). However, under natural circumstances, bulbuls laid a median clutch size of two eggs, whereas median clutch size for broods in both treatments was three.

Table 2: Statistical results from a generalized linear model with Poisson error exploring the difference in clutch size between the experimental treatments (predator-control) and the natural situation.

Predictor	Estimate $\pm$ SE	z-value	Pr(> z )
Intercept (Natural situation)	8.473e-01 $\pm$ 2.182e-01	3.883	0.000103 *
Control treatment	2.256e-11 $\pm$ 4.364e-01	0.000	1.000000
Predator treatment	6.899e-02 $\pm$ 3.381e-01	0.204	0.838288

$N_{Control}(3)$ ,  $N_{Predator}(6)$ ,  $N_{Natural\ situation}(9)$

### Egg size

In the natural situation, the mean egg volume decreased with laying order. For the nests receiving the control treatment, we found the opposite, where mean egg volume increased with laying order. Dark-capped bulbuls that received the predator treatment did not change their investment in the second egg compared to the intra-brood control (first egg).

There was a significant difference in overall mean egg volume between the control treatment and the natural situation (Table 3<sub>(2)</sub>) and between treatments (control – predator) ( $M \pm S.E. = 3158.871 \pm 139.766$ ,  $t_{24.9} = 2.554$ ,  $p = 0.0172^*$ ). Females exposed to playbacks of non-predatory species laid, on average, significantly smaller eggs than birds exposed to an increased perceived predation risk and the natural situation. Whereas mean egg volume does not differ significantly between the predator treatment and the natural situation.

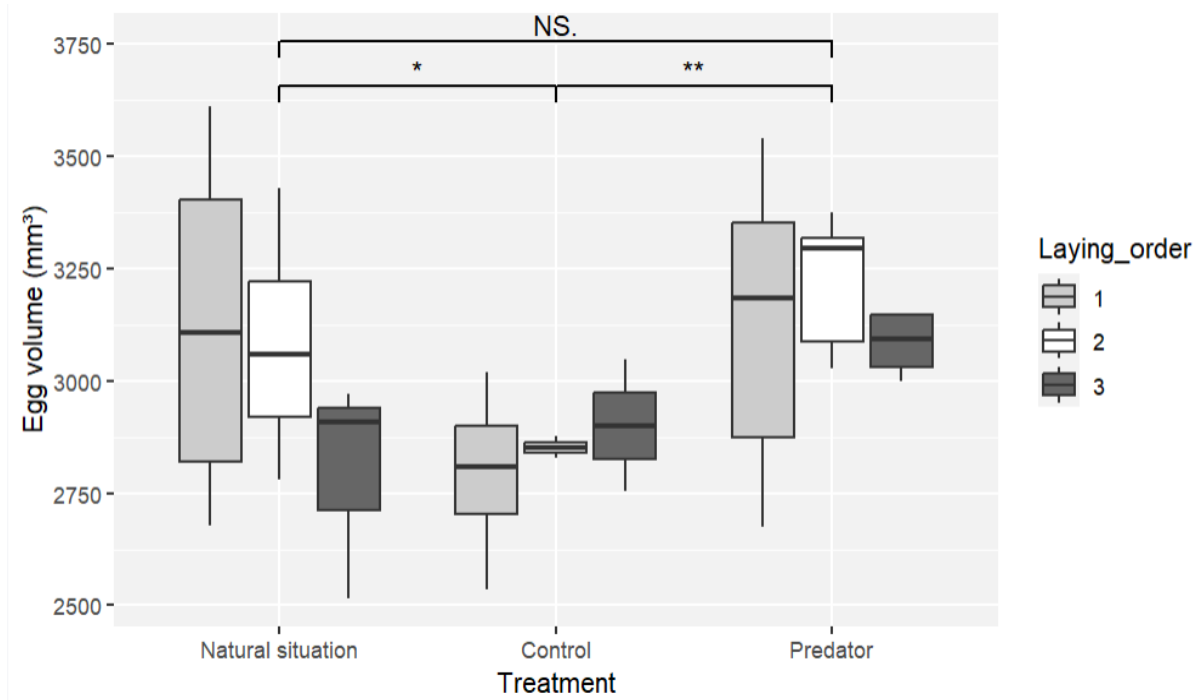


Figure 4: Boxplot showing egg size in relation to laying order (1-2-3), per treatment (control (N=4) - predator (N=9) - natural situation (N=14)). The treatments are depicted on the x-axis, and on the y-axis the egg volume is shown. Per treatment, the results are shown per laying order of the egg with light grey depicting the first egg, white depicting the second egg and dark grey depicting the third egg of the clutch. Significance indicators show significant differences between the natural situation and control, and between the treatments (control-predator) where \* means  $p < 0.05$ , and \*\* means  $p < 0.01$ .

Table 3: Statistical results of two general linear mixed models where we examined possible predictors for a difference in egg volume. The full model (1) uses treatment, laying order, predator density and an interaction between laying order and treatment as fixed factors, and nest id as a random factor. Due to the insignificance of some predictors (predator density and the interaction term between laying order and treatment), we used a reduced model (2) for further statistical analyses.

<b>Predictor</b>	<b>Estimate <math>\pm</math> SE</b>	<b>t-value</b>	<b>Pr(&gt; t )</b>
<b>Full model<sub>(1)</sub></b>			
<i>Intercept</i> <i>(Laying order 1)</i> <i>(Natural situation)</i> <i>(Laying order 1 : Natural situation)</i>	2948.891 $\pm$ 143.402	20.564	<0.001*
<i>Laying order 2</i>	-31.987 $\pm$ 56.291	-0.568	0.5748
<i>Laying order 3</i>	-76.823 $\pm$ 106.039	-0.724	0.4746
<i>Control treatment</i>	-343.637 $\pm$ 141.193	-2.434	0.0207*
<i>Predator treatment</i>	5.508 $\pm$ 106.403	0.052	0.9590
<i>Predator density</i>	6.791 $\pm$ 5.468	1.242	0.2257
<i>Laying order 2 : Control treatment</i>	50.341 $\pm$ 153.493	0.328	0.7453
<i>Laying order 3 : Control treatment</i>	143.447 $\pm$ 177.502	0.808	0.4255
<i>Laying order 2 : Predator treatment</i>	90.346 $\pm$ 96.181	0.939	0.3560
<i>Laying order 3 : Predator treatment</i>	102.148 $\pm$ 143.543	0.712	0.4825
<b>Reduced model<sub>(2)</sub></b>			
<i>Intercept</i> <i>(Laying order 1)</i> <i>(Natural situation)</i>	3089.260 $\pm$ 64.100	48.193	<0.001*
<i>Laying order 2</i>	-2.715 $\pm$ 40.848	-0.066	0.9474
<i>Laying order 3</i>	-10.448 $\pm$ 59.385	-0.176	0.8614
<i>Control treatment</i>	-287.252 $\pm$ 132.117	-2.173	0.0394*
<i>Predator treatment</i>	69.665 $\pm$ 97.700	0.713	0.428

$N_{Control(4)}$ ,  $N_{Predator(9)}$ ,  $N_{Natural\ situation(14)}$

## Nest attentiveness

Dark-capped bulbul pairs exposed to the predator treatment did not differ in their nest attentiveness from pairs receiving the control treatment. Additionally, our results indicate no significant change in the number of visits to the nest per hour (Table 4<sub>(1)</sub>, Figure 5<sub>(a)</sub>), or the proportion of time the parents spent guarding the nest (Table 4<sub>(2)</sub>, Figure 5<sub>(b)</sub>).

Table 4: Statistical results from two general linear models that looked at (1) the number of visits to the nest per hour during the egg-laying period, and (2) the proportion of time spent guarding the nest between experimental treatments (predator-control).

Predictor	Estimate $\pm$ SE	t-value	Pr(> t )
<b>Visits to the nest<sub>(1)</sub></b>			
Intercept (Control treatment)	0.7693 $\pm$ 0.2731	2.817	0.0183*
Predator treatment	-0.2890 $\pm$ 0.3154	-0.916	0.3811
<b>Nest guarding<sub>(2)</sub></b>			
Intercept (Control treatment)	29.237 $\pm$ 11.071	2.641	0.0247*
Predator treatment	-9.541 $\pm$ 12.783	-0.746	0.4726

$N_{Control}(3)$ ,  $N_{Predator}(9)$

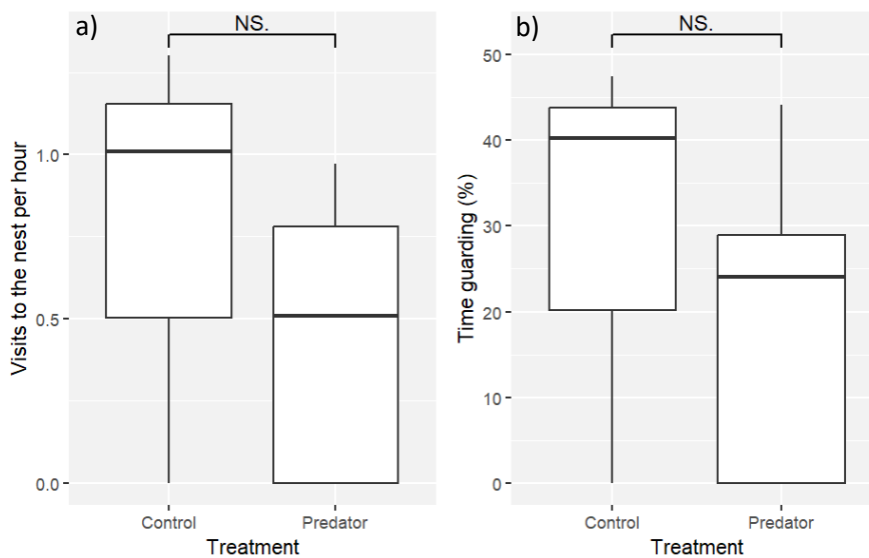


Figure 5: Boxplots showing nest attentiveness, with boxplot (a) indicating a difference in the number of visits to the nest per hour between the experimental treatments (control ( $N=3$ ) - predator ( $N=9$ )). Treatments are depicted on the x-axis, and visits to the nest are shown on the y-axis. Significance indicators show no significant difference between visits to the nest per hour between treatments. Boxplot (b) indicates a difference in the proportion of time spent guarding the nest. Treatments are depicted on the x-axis, and the proportion of time spent guarding the nest is shown on the y-axis. Significance indicators show no significant difference in the proportion of time spent guarding the nest between treatments.

## Incubation behaviour

Pairs of the dark-capped bulbul that received the predator treatment during egg laying showed a similar number of visits to the nest as pairs that were not exposed to the experiment, as our results show no significant difference (Table 5<sub>(1)</sub>, Figure 6<sub>(a)</sub>). Furthermore, our results indicate that females did not change the proportion of time spent incubating when previously exposed to the predator call playbacks (Table 5<sub>(2)</sub>, Figure 6<sub>(b)</sub>).

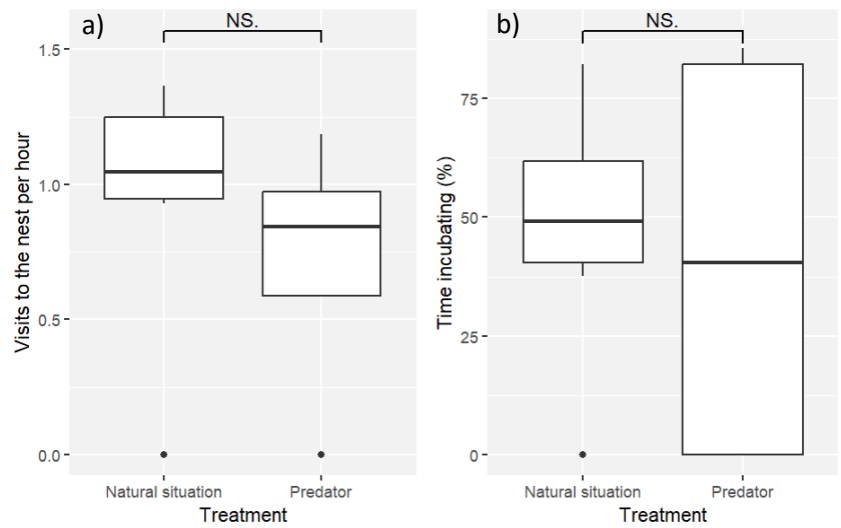


Figure 6: Boxplots showing incubation behaviour, with boxplot (a) indicating a difference in the number of visits to the nest per hour between the predator treatment ( $N=2$ ) and the natural situation ( $N=6$ ). Treatments are depicted on the x-axis, and visits to the nest are shown on the y-axis. Significance indicators show no significant difference in visits to the nest per hour between predator treatment and natural situation. Boxplot (b) indicates a difference in the proportion of time spent incubating. Treatments are depicted on the x-axis, and the proportion of time spent guarding the nest is shown on the y-axis. Significance indicators show no significant difference in the proportion of time spent incubating between predator treatment and the natural situation.

Table 5: Statistical results from two general linear models that looked at (1) the number of visits to the nest per hour during incubation, and (2) the proportion of time spent incubating between experimental treatments (predator-natural situation).

Predictor	Estimate $\pm$ SE	t-value	Pr(> t )
<b>Visits to the nest</b> <sub>(1)</sub>			
Intercept (Natural situation)	0.9471 $\pm$ 0.2036	4.652	0.00164*
Predator treatment	-0.2301 $\pm$ 0.3219	-0.715	0.49505
<b>Incubation</b> <sub>(2)</sub>			
Intercept (Natural situation)	58.18 $\pm$ 48.12	1.209	0.313
Predator treatment	16.54 $\pm$ 53.80	-0.307	0.779

$N_{\text{Predator}} (2), N_{\text{Natural situation}} (6)$

## Discussion

### Physiological adaptations to increased perceived predation risk

Life history theory states that birds should reduce their investment in current offspring under high predation pressure (Eggers et al., 2006; Lima, 2009; Martin & Briskie, 2009; Slagsvold, 1982, 1984; Slagsvold et al., 1984; Travers et al., 2010; Zanette et al., 2011). Moreover, Fontaine & Martin (2006) found an increase in parental investment upon removal of predation risk, so we would expect the opposite to be true with added predation risk, resulting in smaller clutches that hold smaller eggs (Eggers et al., 2006; Fontaine & Martin, 2006; Hua et al., 2014; Martin et al., 2000; Slagsvold, 1984; Slagsvold et al., 1984). However, contrary to what we hypothesised, clutch size remained stable between treatments with most clutches holding two to three eggs. In addition, females did not lay smaller eggs when faced with increased perceived predation risk, as our results did not show a significant difference in egg size between treatments. Possible explanations as to why our results contradict the life-history theory will be discussed in detail below.

Clutches laid under an increased perceived predation risk held as many eggs as clutches laid under our control treatment and the natural situation. Our results are in line with multiple studies that found no change (Fontaine & Martin, 2006; Martin & Briskie, 2009; Massaro et al., 2008; Zanette et al., 2011), although it contradicts the reduction in clutch size that Eggers et al. (2006) and Hua et al. (2014) observed in Siberian jays and Eastern bluebirds (*Sialia sialis*) respectively. Perhaps the discrepancy between our study and what Eggers et al. (2006) found is the most noteworthy comparison to make. Upon increasing the perceived predation risk in Siberian jays with predator call playbacks, Eggers et al. (2006) did find a reduction in clutch size. The main difference between our studies is the dissimilarity in the reproductive strategy of our study species. Dark-capped bulbuls are southern-African passerines that experience a naturally high nest predation risk, have small clutches with innately small eggs and renest multiple times during the breeding season (Hockey et al., 2005; Liversidge, 1970; Martin, 1996; Martin et al., 2006; Sinclair et al., 2020; Tarboton, 2011); whereas Siberian jays are temperate species with a generally lower predation risk that rarely renest (Eggers et al., 2006; Fontaine & Martin, 2006; Martin et al., 2000; Travers et al., 2010; Zanette et al., 2006). Perhaps the decision of clutch size is made well before the start of the current breeding attempt in sub-tropical passerines, and is based on a multitude of external and internal factors; this may explain why the effect of predator playbacks during oogenesis of the second egg on clutch size is rather limited.

Dark-capped bulbuls did not seem to adapt their investment in egg size under increased perceived predation risk. In the untreated nests, we found a minor, yet insignificant, reduction in egg size with laying order, contrary to what Slagsvold et al. (1984) found in passerines in temperate areas. Dark-capped bulbuls potentially adopt the brood-reduction hypothesis, which states that egg size decreases with laying order (Arnold, 1991; Slagsvold et al., 1984). The eight species in the study of Fontaine & Martin (2006) that showed plasticity by increasing egg size upon eradicating predation risk, were all temperate passerines found in North America. Again, these temperate passerines are known to adopt different reproductive strategies than sub-tropical passerines (Martin, 1996; Martin et al., 2006; Slagsvold et al., 1984). Whereas life-history theory predicts even smaller eggs with increased predation risk, it might be more viable for birds to forsake breeding altogether than to lay poor-quality eggs (Lima, 2009). This might explain why we did not observe a reduction in egg size when increasing the risk of their current clutch getting predated. Moreover, more tropical regions such as the southern-African savanna, have much less seasonality than temperate regions. Hence, a decreased seasonality could lead to a longer breeding season and thus more renesting attempts; as well as an overall higher adult survival rate (Martin, 1996). The breeding season of the dark-capped bulbul is spread over several months, starting in September and ending mid – February with a peak between October and December. As discussed earlier, they have multiple breeding attempts during

one breeding season (Hockey et al., 2005; Tarboton, 2011). Furthermore, dark-capped bulbuls have an average adult survival of over 74% (Hockey et al., 2005), which is considerably higher than the mean survival rate in temperate passerines in Europe (52%) and in northern America (53%) (Johnston et al., 1997). This difference in seasonality, length of breeding season and adult survival rate in sub-tropical passerines favours a reduced total reproductive effort in sub-tropical bird species (Martin, 1996), and could be yet another potential reason as to why we did not find a reduction in egg size. Thus, it has become clear that sub-tropical passerines do not adopt similar plasticity in egg size when faced with an increase in predation risk as temperate species.

Despite our results indicating no change in clutch size, the dark-capped bulbul is known to alternate between a clutch of two or three eggs (Hockey et al., 2005; Sinclair et al., 2020; Tarboton, 2011), which is a considerable difference in overall energy expenditure (Fontaine & Martin, 2006). Perhaps a singular adaptation of egg size under increased predation risk can yield long-term reproductive benefits for the female. Despite our results showing no effect of our treatments on egg size, if we look carefully there is a slight (7,5%) increase in the size of the egg that was exposed to increased perceived predation during oogenesis. However we know that this is not a significant biological trend we replicated, we would like to look at this slight increase as a tendency. The tendency to increase egg size could indicate that dark-capped bulbuls invest more resources in their eggs under increased predation risk. Adaptations such as increased clutch size, nest attentiveness and incubation behaviour demand long-term and repeated energetic input; whereas an increase in egg size requires relatively limited input (Fontaine & Martin, 2006). This one-time increase in egg size results in well-developing chicks upon hatching, allowing the parents to decrease nest visits and maintain nest crypsis, thus reducing predation risk (Hargitai et al., 2005; Zanette et al., 2011). However, if the tendency to increase egg size under a heightened predation risk is a projection of a biological trend, the question remains: if nest predation is higher during the egg-laying period, as stated by Lloyd (2004) and Martin et al. (2000), why invest more energy in a larger egg that has a higher chance of getting predated?

The reason we might have failed to replicate clear biological trends, found by other studies, could be nested in the decisions we made to include or exclude certain nests in our analyses. Although not described in literature, we included clutch sizes of one egg in our dataset and thus our results. In the field, we encountered clutches that once had multiple eggs, got predated, and had a single egg remaining. Parents did not abandon the nest and continued their current reproductive attempt with the remaining egg. The same is true for clutches with two eggs. With no predation event observed, we cannot say with certainty what the actual intent of the bird was before starting the current clutch. Additionally, the unusually small egg volume of the first egg in the control treatment stands out compared to the other two treatments. Due to our experimental set-up, the first-laid egg served as an intra-clutch control and thus depicted the egg-laying under natural conditions. We unknowingly integrated nests with very small eggs in our control treatment, and due to the small sample size, this deviation becomes apparent, skewing our results. Perhaps an overall larger sample size could yield a different, significant outcome since having a greater number of nests and thus eggs, might mitigate the effect of outliers that possibly have a larger effect in our current study, causing us to find a mere tendency.

#### Behavioural adaptations to increased perceived predation risk

Most previous studies hypothesised and reported an overall reduction of parental activity near the nest with increased predation risk, expressed as less frequent visits to the nest, and less time spent near the nesting site during egg laying or longer on-and-off bouts during incubation (Basso & Richner, 2015; Conway & Martin, 2000; Fontaine & Martin, 2006; Ghilambor & Martin, 2002; Martin, 1996; Martin & Briskie, 2009; Martin et al., 2000; Massaro et al., 2008; Morosinotto et al., 2013; Zanette et al., 2011). However, parents do not alter their nest attentiveness or incubation behaviour upon



exposure to increased perceived predation risk during egg-laying. Below, we provide insights into why dark-capped bulbuls do not seem to behave according to the life history theory.

As our results showed, dark-capped bulbuls preferred to stay close to the nest, even under increased predation risk. A possible explanation is that both parents choose to remain in the vicinity of the nest enabling them to fend off potential predators, as we observed in one of our recordings. A pair of bulbuls was seen attacking a juvenile boomslang (*Dispholidus typus*) preying on their nest. It is possible that the main nest predators of savanna passerines are not visually oriented (e.g. birds and mammals); but olfactory oriented predators (e.g. snakes and monitors) (Lloyd, 2004). This makes it unyielding for the parents to decrease the activity near the nest, as it does not greatly affect predation probability. Another interesting finding by Basso & Richner (2015) could be a possible explanation for the lack of reduction in the duration of incubation when previously exposed to predator calls. Under an increased perceived predation risk during the day, females increased their incubation length during the night (Basso & Richner, 2015). We cannot make any inferences about this, as we did not collect data on nocturnal incubation behaviour. Additionally, nesting site selection and vegetation density might play a role as well. Possibly dark-capped bulbuls strategically choose their nesting site, ensuring that the vegetation provides sufficient camouflage for their nest to remain undetected, thus lowering the chances of predation. Hence, not directly responding with a decrease in presence near the nesting site, might prove advantageous for dark-capped bulbuls.

We did however encounter some plausible patterns that might indicate that bulbuls do adjust their behaviour to predation risk. Despite our non-significant result, the proportion of time spent guarding the nest decreased from slightly over 40% during playbacks of non-predatory species, to less than 25% when predator playbacks were played. This is a reduction of nearly half of the time spent guarding the nest. Besides, without any additional playbacks played during incubation, we see a minor yet insignificant reduction in time spent incubating in nests previously exposed to the predator treatment alongside a slight decrease in visits to the nest, contrary to what life-history theory predicts. This is similar to what Zanette et al. (2011) and Morosinotto et al. (2013) found in song sparrows and pied flycatchers (*Ficedula hypoleuca*) respectively. A possible explanation as to why birds would reduce their visits to the nest under increased predation risk is that the chance of nest predation is higher during the egg stage of the breeding cycle (Lloyd, 2004; Martin et al., 2000). Thereby, the majority of predators predate on eggs rather than on nestlings. It might therefore be advantageous to decrease the number of nest visits during the egg stage. Whereas a decrease in both visits to the nest and time spent incubating could be advantageous to not lure predators near the nesting site (Conway & Martin, 2000; Ghalambor & Martin, 2002; Ghalambor et al., 2013; Martin, 1996; Martin & Briskie, 2009; Morosinotto et al., 2013; Zanette et al., 2011), it prolongs the time before hatching, putting the current clutch at risk for a longer period. According to Ghalambor & Martin (2002), this is especially relevant for species with an open cup nest that nest in areas with high predator densities, such as the dark-capped bulbul (Ghalambor & Martin, 2002; Martin & Briskie, 2009). It has become clear that a change in parental behaviour under predation pressure is ambiguous and can be highly variable depending on the situation and the intrinsic value of the current brood.

The observed reduction in nest attentiveness and incubation behaviour, however insignificant, might be an indication of a trend that we possibly failed to replicate in our experiment due to methodological issues. These methodological issues include, for example, a high predation rate on our nests, technical issues and human errors that ultimately resulted in a limited sample size. The spread of the data in the predator treatment is quite broad because some nests most likely got abandoned and showed no birds in the incubation footage. Additionally, no nests receiving the control treatment survived until the incubation stage and only two nests receiving the predator treatment made it to incubation. This again designates the very high predation rate in the Mbuluzi Game Reserve, where only 28% of dark-capped bulbul nests fledged successfully (Eshuis, 2023; Martin, 1996; Martin et al.,

2006). Thus, we conclude that increasing our sample size will diminish methodological issues as well as act as a buffer for the high natural predation rate, therefore providing a better insight into the tendencies we found.

#### Changes in parental investment to increased perceived predation risk

From our results, it has become clear that experimentally increasing perceived predation risk does not affect parental investment in the dark-capped bulbul. And surely, many factors contribute to changes in clutch- and egg size, nest attentiveness and incubation behaviour; but because of our experimental setup, we account for the majority of these other factors, making the changes we see ascribable to our treatment.

Perhaps our experimental setup, of solely playing playbacks, did not have a sufficient effect. As the natural predation pressure is high in savanna habitats (Martin, 1996; Martin et al., 2006), it might not display any notable fluctuations. This may have caused the dark-capped bulbul to become habituated to the constant piercing predation pressure, to the extent that only adding bird calls does not affect the investment as much as it does in temperate species. Adding a visual model of the predators, possibly combined with alarm calls from different species, including the dark-capped bulbul, could have a much greater effect. Thereby, it is important to note that during all treatments, predators were still calling and predation still occurred. Maybe if we could eradicate actual predation pressure to some extent, as Martin et al. (2006) did, we might find very different results. The non-responsiveness to predation pressure might however be their strength. Different species of savanna passerines with biparental care systems, such as the brown-crowned tchagra (*Tchagra australis*) or the African paradise flycatcher (*Terpsiphone viridis*), might respond differently to increased perceived predation risk, provided by our experimental setup, due to their taxonomical differences from the dark-capped bulbul. Hence, the remarkably high predation pressure in southern Africa and the sensitivity to it, or lack thereof, of the dark-capped bulbul seems to be an unresolved matter.

Predation might simply not be one of the main drivers of changes in parental investment in savanna passerines (Lima, 2009; Martin & Briskie, 2009; Sarkar & Khajanchi, 2020). Or predation might have shaped parental investment in such a way that the risk of predation is already minimised to its maximum. Additionally, dark-capped bulbuls have more environmental factors to take into account than predation risk, such as parasitism risk. Bulbuls are the main host species to the Jacobin cuckoo (*Clamator jacobinus*) (Hockey et al., 2005; Payne & Payne, 1967; Sinclair et al., 2020; Tarboton, 2011), and perhaps their focus lies more on preventing parasitism than preventing predation. On the other hand, it could be that for more tropical species food limitation and temperature are greater drivers of changes in parental investment, contrary to what was found in temperate species (Martin & Briskie, 2009; Nager & Zandt, 1994; Ojanen et al., 1981; Smith et al., 1989). Thus, what actually affects and shapes parental investment in dark-capped bulbuls is most likely a multifactorial matter, which opens up possibilities for further research.

To summarize, dark-capped bulbuls did not display a clear change in parental investment under an experimentally increased perceived predation risk. Much like in most other temperate passerines, the number of eggs in clutches remained stable for dark-capped bulbuls (Fontaine & Martin, 2006; Martin & Briskie, 2009; Massaro et al., 2008; Zanette et al., 2011), indicating that perhaps the decision of clutch size is rather multifaceted and thus relatively unaffected by predation risk. Unlike the similarity between temperate and sub-tropical passerines in plasticity in clutch size, plasticity in egg size did not behave accordingly. Whereas females of temperate passerines laid smaller eggs under an increase in predation risk, sub-tropical passerines did not change their investment, only showed a mere tendency to increase egg size. When examining parental behaviours under increased predation risk, it became clear that dark-capped bulbuls did not adjust their behaviour according to life-history theory, which was based on temperate passerines. Parents faced with increased predation risk during

egg-laying did not markedly change parental behaviours; they only showed the tendency to decrease the overall activity near the nest, even the duration of incubation bouts. Therefore, with predation risk and associated behavioural adjustments being so dependent on the situation, thus showing no significant results, it becomes perilous to make inferences about this. Hence, we can conclude that parental investment in dark-capped bulbuls is not considerably affected by increased perceived predation risk, contrary to what we predicted.

## Conclusion

In our attempt to fill the knowledge gap considering the main drivers of life-history decisions relating to parental investment in savanna passerines living in sub-tropical climates; we examined the effect of increased perceived predation risk on the clutch - and egg size, nest attentiveness and incubation behaviour in the dark-capped bulbul, whilst controlling for ambient predation risk. Despite our predictions, based on life-history theory, we did not find similar physiological and behavioural adaptations to predation risks in savanna passerines as seen in temperate passerines. However, we did observe some tendencies that could be indicative of biological trends that we were unable to portray in our study. Perhaps dark-capped bulbuls simply are not able to, or do not feel the need to display plasticity in parental traits under increased predation risk. This again illustrates that parental investment strategies vary across climates and are contingent on how much such strategies were already optimised as a response to natural circumstances. As a take-home message, we want to emphasise that determining what shapes life-history decisions and therefore traits, is not always straightforward. Nonetheless, to further advance our knowledge, it remains important to keep questioning and researching these life-history questions in an array of species and taxa across different biomes.

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## Appendix

Table 6: Daily fieldwork planning.

Daily fieldwork planning*	
05.00h – 09.00h	Checking empty bulbul nests for first eggs. When the first egg is found: start experiment. Setup incubation watches.
09.00h – 12.00h	Nest searching. Weighing and ringing of 9-day-old chicks.
13.00h – 17.00h	Data entry. Downloading of video footage. Planning for the next day.

\*times can differ per day and workload.

Table 7: Fieldwork planning per nest, per day.

Fieldwork planning per nest	
Day 0	An empty active bulbul nest is found.
Day 1	The first egg is found, measured and marked. The nest is randomly assigned to a treatment. A speaker is placed and playbacks are played (between 05.00 and 09.00). A camcorder records nest attentiveness (between 05.00 and 17.00). The speaker and camera are collected (between 16.00 and 17.00).
Day 2	The second egg is measured and marked. Day of clutch completion if the clutch consists of two eggs. Start incubation.
Day 3	The third egg is laid, if there is one. No visit. Day of clutch completion if the clutch consists of three eggs. Start incubation.
Day 6-9*	The camcorder is placed to record incubation behaviour (between 05.00h and 07.00h). The camera is collected (between 16.00h and 17.00h).

\*flexibility in day of placement due to weather restrictions.