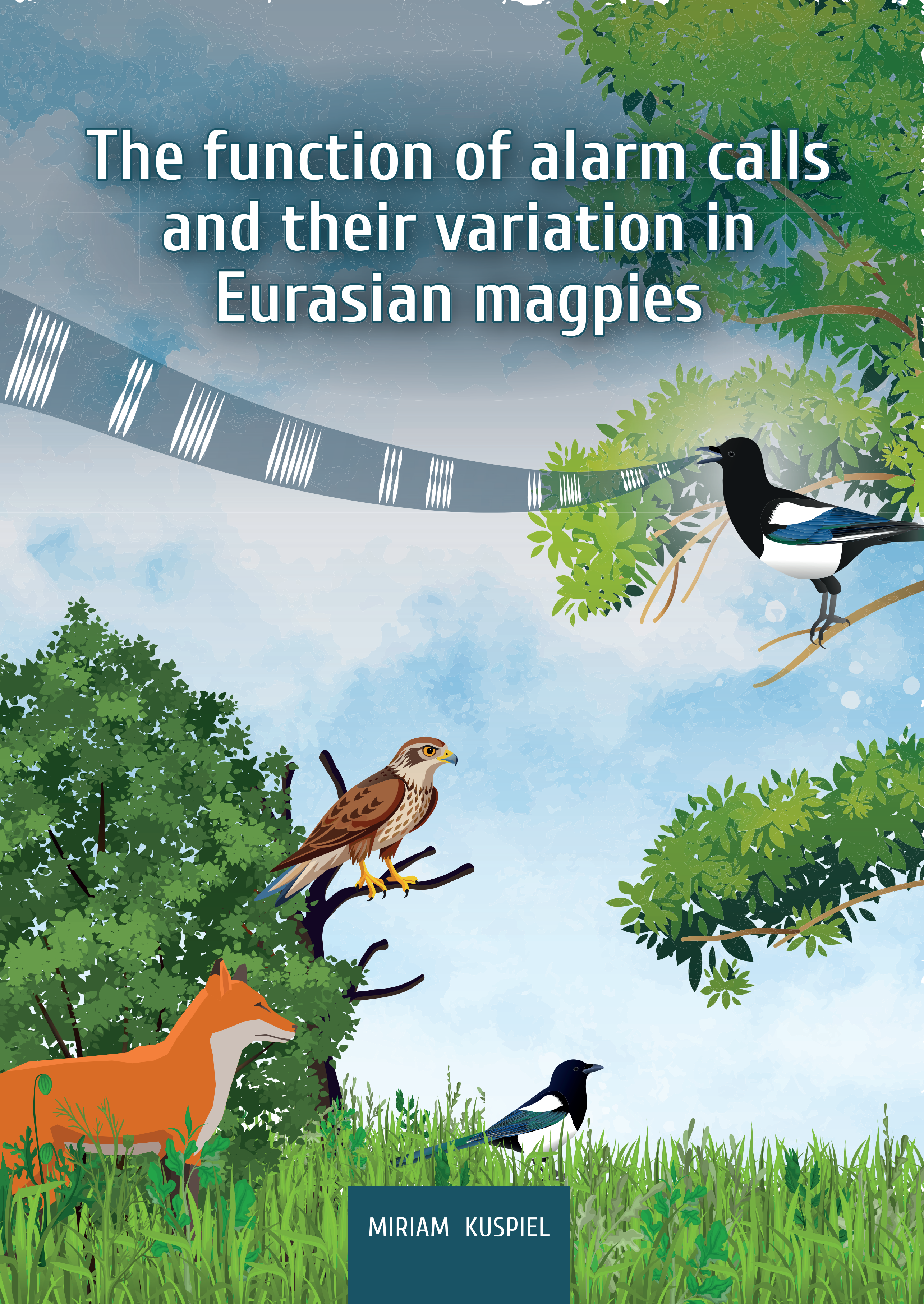
The function of alarm calls and their variation in Eurasian magpies



MIRIAM KUSPIEL

Propositions

1. The distinction between bird song and calls is poorly defined and outdated.
   (this thesis)

2. Simple calls have complex functions.
   (this thesis)

3. All statistical analyses are subjective.

4. Selection for specific personality traits in scientists harms science.

5. Studying tested hypotheses and studying novel ideas are equally important to fund.

6. Scientific quality is independent of successful academic careers.

7. Flex-desks create a poor work environment.

8. Flying around the world is a luxury that the world cannot afford.

Propositions belonging to the thesis, entitled

The function of alarm calls and their variation in Eurasian magpies

Miriam Kuspiel
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The function of alarm calls and their variation in Eurasian magpies

Miriam Kuspiel

Thesis Committee

Promotor

Prof. Dr Marc Naguib
Professor of Behavioural Ecology
Wageningen University & Research

Co-promotor

Dr Sjouke A. Kingma
Associate Professor, Behavioural Ecology Group
Wageningen University & Research

Other members

Prof. Dr Marcel Dicke, Wageningen University & Research
Dr Claudia Fichtel, German Primate Center, Germany
Dr Michelle J. Spierings, Leiden University
Prof. Dr Mathieu Amy, Université Paris Nanterre, France

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The function of alarm calls and their variation in Eurasian magpies

Miriam Kuspiel

Thesis

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Miriam Kuspiel

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Ch. 1 | General introduction

(1.1) The study of communication complexity

The high complexity of languages to communicate in great detail is unique to humans (Fedurek & Slocombe 2011), yet complex communication through acoustic signals is widespread across the animal kingdom. Animals from insects such as crickets, amphibians such as frogs, birds and mammals, but also fish and reptiles produce acoustic signals (Smith 1977; Suthers *et al.* 2016). Historically, non-human acoustic signals were thought to be merely an expression of the internal state or characteristics of an individual (Smith 1977). In the last decades, however research has found similarities between non-human acoustic signalling and human speech (Rendall *et al.* 2009; Fedurek & Slocombe 2011). Animals like songbirds and primates have been studied extensively to understand the development and learning of vocal signals and the likely evolutionary drivers of complex communication systems culminating in human languages (Papoušek *et al.* 1992; Doupe & Kuhl 1999; Seyfarth & Cheney 2010).

To understand the meaning and function of signals, substantial research focussed on how vocalisations are used in different contexts. Bird song was among the first complex signals to be studied in much detail, and was quickly identified as mainly a sexually selected signal functioning in female choice and male-male competition for mates and territories (Barrington 1773; Searcy & Nowicki 2000; Marler 2004; Catchpole & Slater 2008). There exist many examples for highly complex song structures (e.g. nightingales *Lucinia megarhynchos* (Todt 1971; Kipper *et al.* 2004), lyrebirds *Menuridae* (Robinson & Curtis 1996; Dalziell *et al.* 2021), northern mockingbirds *Mimus polyglottos* (Derrickson 1987; Roeske *et al.* 2021)). Still, signal variation is commonly associated mainly with intrinsic features of the caller, such as the species, the caller's identity, quality, age, motivation or aggressiveness (Vehrencamp 2000; Gil & Gahr 2002; Catchpole & Slater 2008). The latter may further predict the likelihood of subsequent behaviour such as attack or withdraw (Searcy & Beecher 2009; Naguib & Mennill 2010; Bradbury & Vehrencamp 2011). In other contexts, parallels have been drawn between animal calls and human words: In several species, specific meanings like "attention, raptor" or "hide", have been associated with specific calls (Macedonia & Evans 1993; Gill & Bierema 2013; Suzuki 2016). Going even further, various studies explored how the function of acoustic signals depends on the combination with other calls or even the order in which calls are emitted, drawing comparisons to syntax in human speech (Hailman & Ficken 1986; Griesser *et al.* 2018; Engesser & Townsend 2019). While all these examples of seemingly highly complex communication are very fascinating, many species do not use such complex vocal communication or produce general calls across a broad range of contexts (Dezecache & Berthet 2018). Especially the closest relatives of humans, great apes, appear to be constrained in a rather small and “innate” vocal repertoire and lack predator-specific alarm calls (Wheeler & Fischer 2012). How can we infer meaning and function of such general calls, and is the communication system truly as invariable as it seems?

In my thesis, I explore the function and complexity of the seemingly simple “chatter” alarm call in Eurasian magpies (*Pica pica*). These corvids, like great apes, are described to possess high cognitive abilities (Pollok *et al.* 2000; Prior *et al.* 2008). Moreover, they belong to the passerine family which comprise prominent examples of high song complexity (e.g. nightingales, Kipper et al., 2004; Todt, 1971). Like few other passerines, magpies can also mimic sounds (Baeyens 1979). Other members of the corvid family, the Siberian jays *Perisoreus infaustus* (Griesser 2008, 2009), are further known to have context-specific calls for different predator types and predator behaviours. Yet, despite all these indications that magpies should have a complex communication system, they actually appear to produce the same “chatter” call (a loud trill of short, harsh, broad-band notes) across a variety of predatory and conspecific contexts (Birkhead 1991). I therefore investigate the function of chatter variation in this thesis to explore if it is truly as invariable as it seems.

(1.2) The meaning of calls

Various lines of research have identified different types of information that can be encoded in acoustic signals:

First, vocal signals can encode information about traits of the caller, such as what species it is (Charlton *et al.* 2020). In the dawn chorus of birds, for example, different species can be readily identified based on their song, which enhances chances of finding a mate and determines location of competitors (Becker 1982; Catchpole & Slater 2008). Many vocalisations also enable individual identification (Wiley 2013), so that, for example, mothers in colonial breeders can find their own offspring or partner (e.g. cliff swallows *Hirundo pyrrhonota* (Stoddard & Beecher 1983), various pinniped species (Insley *et al.* 2003), penguins (Aubin & Jouventin 2002)). Many bird species can also discriminate between vocalisations of neighbours and strangers (McGregor & Avery 1986; Godard 1991; Tibbetts & Dale 2007). In group-living species, information about the sex, dominance status and group membership may also be embedded in vocalisations (Fischer *et al.* 2004; Manser 2017). Vocal signatures need not be constant within individuals but may vary with hormonal status (e.g. indicating reproductive states), or health condition (Duffy & Ball 2002; Schmidt *et al.* 2014). Such information content, however, is likely an intrinsic feature that is not manipulated intentionally by the caller.

Second, vocalisation features may relate to the motivational or emotional state of the caller: Darwin originally suggested that vocalisations are mainly automatic expressions of emotions, which may be associated with certain functions secondarily (Darwin 1872/1998). Certain features of vocalisations, for example the frequency measures, can indeed be associated with the emotional states or stress experienced by the caller (Fichtel *et al.* 2001). Later, Smith (Smith 1977) defined calls to be motivational and to reflect, for example, the arousal of an individual, so that its likelihood to attack can be inferred. Still, these signals are mainly thought to refer to the internal state of the individual, e.g. its willingness to mate, its aggression towards competitors, or its arousal in agonistic interactions or in face of a predator.

Third, in contrast to these former definitions, acoustic signals were suggested to refer to external objects or events (Marler *et al.* 1992; Macedonia & Evans 1993). By determining the context in which distinct vocalisations were emitted, researchers inferred a meaning of these calls. One of the first were Struhsaker's very detailed descriptions of vervet monkey (*Chlorocebus pygerythrus*) calls, which he categorised as agonistic, as predator-related, as distress-on-separation or as appeasement calls, with different functions therein (Struhsaker 1967). Studies on vervet monkeys have been continued in more standardised ways thereafter. In order to be defined as a "referential signal", calls must firstly be context-specific (Macedonia & Evans 1993). Indeed, vervet monkeys were shown to

Glossary

alarm call – a call given in a predatory context (Klump & Shlater 1984, Zuberbühler 2009)

context-specific call – a call that is produced only in specific contexts; depending on the range of contexts, a call can be less or highly context-specific (i.e. vary in referential specificity, also called production-specificity) (Macedonia & Evans 1993, Wheeler & Fischer 2012)

functional referential call – a call that fulfils high context-specificity and perception-specificity (Macedonia & Evans 1993)

general alarm call – alarm call that is emitted towards a broad range of disturbances / in a range of potentially threatening or arousing contexts, i.e., has low context-specificity (Dezecache & Berthet 2018)

graded variation – variation within a call type or how it is emitted, for example variation in call duration, call frequency, or call rates (Morton 1982, Suzuki 2016)

(continued on next page)

produce distinct calls for different types of predators, i.e. mammalian carnivores, raptors and snakes (Seyfarth *et al.* 1980b), though later findings questioned the predator-specificity (Price *et al.* 2015; see below). Next, referential signals must have perception specificity, meaning that the call elicits the same response as the threat it stands for, and thus truly informs about the presence of a predator or tells receivers to behave in a certain way (Macedonia & Evans 1993). In case of the vervet monkey, the different types of predators as well as the corresponding calls alone were shown to elicit running up a tree on hearing 'leopard' calls, looking up and hiding in a bush on hearing "eagle" calls in case of the vervet monkeys (Seyfarth *et al.* 1980b, a). Henceforth, multiple studies revealed that a variety of species produce at least some context-specific and perception-specific signals for certain predators (Evans *et al.* 1993; Gill & Bierema 2013; Suzuki 2016; Smith 2017). In addition, referential food calls can inform receivers about the presence of food (Clay *et al.* 2012; Smith 2017). Certain vocalisations are even specific to the behaviour of the predator: Since the danger level of a raptor varies when in flight versus on the ground or perched, the vocalisations and responses of its prey may also vary from escapes when the raptor is flying to mobbing (i.e. approaching the predator and calling at it to chase it away) when stationary (Griesser 2008; Cunningham & Magrath 2017). To go further and test if these calls are really associated with mental images, studies by Suzuki and colleagues (Suzuki 2018, 2019) have shown that great tits (*Parus major*) specifically search for snakes on hearing the "snake" alarm calls and are more likely to spot and alarm about a snake-like stimulus. Overall, there is ample evidence for specific calls in a variety

mobbing call – alarm call that is emitted while mobbing a predator, which is (i) directed at the predator to repel it, and (ii) potentially directed at conspecifics to alert and recruit them to mob the predator (Carlsson & Griesser 2022, Zuberbühler 2009)

perception-specific call – a call that elicits a specific response that is appropriate for the call-eliciting stimulus (e.g. escaping on hearing a predator-specific call) (Macedonia & Evans 1993)

predator mobbing – prey approaching or harassing a predator, i.e. usually a group of individuals of one or multiple species assemble around a predator and display at it with visual wing and/or tail movements and loud calls with usually a broad frequency spectrum, possibly involving flights or attacks at the predator (Carlsson & Griesser 2022, Curio 1978)

warning call – alarm call that functions as vocal warning signal to alert conspecifics of a potential danger and may elicit the appropriate escape or freeze response (Klump & Shalter 1984) of species to refer to external referents, such as predators.

While, in the previous examples, specific call types were associated with specific contexts, also graded variation within calls may contain information about external events or internal states (Marler *et al.* 1992; Blumstein 2007; Suzuki 2016). Early studies on various ground squirrels (*Sciuridae*) failed to find predator-specific alarm call types, supposedly because predators usually elicit a similar response, i.e. fleeing to the next burrow entrance (Blumstein 1999b). Thus, there would be no need to have specific call types for different predators. They did find, however, that alarm calls varied gradually, e.g. varied in the rate or frequency at which they were emitted (Blumstein & Armitage 1997). Some of this variation correlated with the distance of the predator from the caller, or even the danger level a predator poses (Blumstein 1999a). Playbacks of variants associated with greater danger or urgency partially elicited a stronger response than playbacks of lower-urgency variants, demonstrating the perception-specificity of graded variation (Blumstein 1999a). Further studies, for example in meerkats (*Suricata suricatta*), have shown that even signals specific to certain predator types can vary with the distance of a predator, i.e. the urgency of a threat (Manser 2001). Multiple mechanisms (call types and variation within call types) can thus convey information about the type or urgency of an external event.

That vocalisations are associated with external referents, however, does not exclude that motivational factors explain why or how animals vocalise (Marler *et al.* 1992). Especially for graded variation, it cannot be distinguished if individuals increase their call rate in order to signal to receivers that a threat is more or less urgent, or purely due to their own arousal level, which likely correlates with, for example, the distance of a predator (Marler *et al.* 1992; Suzuki 2016). Also in humans, differences in the pitch or amplitude of, for example, saying or shouting out "help", are likely caused by the state of the caller (Filippi *et al.* 2017; Palo 2020). Still, receivers likely respond faster or more likely to a highly distressed signal, and thus make use of the information conveyed in the call characteristics to learn how great or urgent a threat is, even if that was not necessarily the intention of the caller (Morton 1977; Marler *et al.* 1992). Regardless of whether or not a signal refers to internal referents (about the caller) or external referents, it can contain a variety of information. We can only understand such information if we explore also broad and subtle variations in contexts in which vocalisations are emitted.

Despite the many ways in which information can be conveyed in acoustic signals, vocalisations often appear to be general calls without high context-specificity (Dezecache & Berthet 2018; Fichtel 2020). One explanation may be the lack of need for specialised calls: As hypothesised for ground squirrels, different call types would only be needed if the response to the predator differs (Blumstein 1999b). It is thus important to explore also non-vocal behaviours to different threat types. Also, the urgency of a threat plays a role for the behavioural response to threats: if various threats do not necessarily require a very fast response, then a general signal that alerts conspecifics may suffice, so that individuals use other contextual cues to identify the cause of the call and respond based on the integrated information (Wheeler & Fischer 2012; Dezecache & Berthet 2018; Fichtel 2020). And even the vervet monkeys, the textbook example for referential calling, were later shown to produce some "predator-specific" calls also in other contexts, such as conspecific interactions (Price *et al.* 2015). In order to understand what drives the specificity of call types or complexity of a communication system, we need to a) cover a variety of contexts and study alarm calls and variations therein not only in predatory contexts, since we may overlook their role in other contexts then, and b) investigate a larger variety of species. It does not suffice that we already know for various species that they produce signals with specific meanings or meaningful variation, or that others seemingly only produce very general calls for a variety of contexts; we need a full picture if we are to understand the evolution of vocal signal complexity.

As one step towards understanding the complexity and evolution of vocal communication, I explored the potential meaning of an alarm call in a - so far - little studied species: The Eurasian magpie. Specifically, I studied the context-specificity of the chatter call and graded variation there-in as well as other behavioural responses across different predatory and conspecific intrusion contexts (**Chapter 2**). In addition, I tested the perception-specificity of variations in the chatter call in a territorial intrusion context (**Chapters 4 and 5**).

(1.3) Why call when detecting a predator?

When exploring the potential meaning of calls in the previous section, I already touched upon the function of alarm calls, which are usually assumed to warn conspecifics. If so, alarm calling would only have an advantage if conspecifics are present to perceive the calls. Still, already in the first studies on referential alarm calls in chicken, audience effects were tested, which indicated different call functions (Gyger *et al.* 1986; Karakashian *et al.* 1988; Evans & Marler 1991; Evans *et al.* 1993). When presented with simulated aerial predators, fowl (*Gallus gallus*) would always crouch down, but emit alarm calls only when another individual was in a cage next to it while they remained silent when alone. These observations confirmed that those calls specific to aerial predator detections functions as warning signal to conspecifics. Their observations of alarm calls elicited by racoons, on the other hand, led to a different conclusion (Evans *et al.* 1993; Marler & Evans 1996). Those calls, in contrast to the aerial predator calls, were very loud and the chicken did not try to escape or be inconspicuous to the predator. Moreover, they also called irrespective of the presence of conspecifics. These “racoon” alarm calls thus appear to function not (primarily) as signal towards conspecifics but as signal at the predator, to advertise that it has been spotted and presumably de-motivate attacks (i.e. appear to be mobbing calls). Such “audience effects”, where the likelihood to emit an alarm call depends on the presence of (specific) conspecifics can thus be studied to explore the function of alarm calling (Gyger 1990; Fichtel & Manser 2010).

Still, even when alarm calls function to warn conspecifics, there needs to be a net fitness benefit to the caller, despite the potential risk it is taking (Maynard Smith 1965; Zuberbühler 2006). Emitting an alarm call may be very costly by making the caller more conspicuous to the predator and thus increasing the risk of being attacked (Sherman 1977; Shalter 1978; Caro 2005; Carlson & Griesser 2022), unless predators do not attack individuals that have detected them already (Caro 1995; Bergstrom & Lachmann 2001). Such potential costs have been rarely quantified, yet it seems valid to assume that alarm calls only evolve if they ultimately benefit the caller. A suite of hypotheses have been suggested to explain why individuals emit alarm calls, which depend on the function and intended receiver (addressee) of the call (Zuberbühler 2006; Carlson & Griesser 2022). For example, the kin selection hypothesis predicts that individuals call to warn kin and thereby increase their inclusive fitness (i.e. they gain fitness by protecting individuals that share part of their genes; Hamilton 1964; Maynard Smith 1965). In contrast, the pursuit-deterrence hypotheses predicts that alarm calls are directed at the predator to advertise that they have been perceived and motivate them to move away, thereby increasing the survival chances of the caller (Caro 1995). In **Chapter 3**, I explore the various hypotheses on why Eurasian magpies alarm call by testing the relationship between social environment and magpies alarm call usage.

(1.4) Acoustic signalling during territorial defence

The previous two sections focussed mainly on the meaning and function of vocalisations in predatory contexts, yet acoustic signals are also frequently produced in competition between conspecifics in a variety of taxa (Bradbury & Vehrencamp 2011). Red deer stags (*Cervus elaphus*), for example, fill autumn forests with loud roars as they compete over female harems (Clutton-Brock & Albon 1979). Since acoustic features are associated with a larger body size, weaker competitors may already retreat without physically fighting to save energy and avoid injuries (Clutton-Brock *et al.* 1979; Reby & McComb 2003). Competitors mostly escalate fights only when they are very similar in strength and thus displays cannot resolve who is stronger (Maynard Smith & Price 1973; Clutton-Brock *et al.* 1979; Bradbury & Vehrencamp 2011). For signals to remain honest, signals need to be directly correlated with measures of individual condition, strength, or territory quality, or cheating would need to be costly, for example if cheaters risk suffering retaliation costs or injuries if the fight with a stronger individual escalates (Vehrencamp 2000; Searcy & Nowicki 2010; Bradbury & Vehrencamp 2011). Vocalisations may thus serve as honest signals of strength or motivation to escalate a fight in competitions over territories or mates.

In birds, the most famous and well-studied signal in territorial defence is bird song. Songs are differentiated from calls as being “longer, more complex vocalisations than calls that are mostly only produced by males during the breeding season” (Catchpole, 1982; Catchpole & Slater, 2003). Especially in the Northern hemisphere, mostly males advertise their territory ownership and attract females through loud song, forming the enticing dawn chorus that marks the commence of spring (Catchpole & Slater 2008). Playback removal experiments have tested the function as territorial signal in several species by removing the resident male but broadcasting its song in the territory, which led to the territories with song playback being taken over by a new male later than control territories without song (Krebs 1977; Nowicki *et al.* 1998). These findings are supported by experiments in which males were manipulated to be incapable of singing and consequently were frequently challenged and outcompeted by other, unmuted males (Smith 1979; McDonald 1989). Hence, bird song clearly has a major function in territorial defence and competitive interactions in a many (though not all) species (Loning *et al.* 2023).

Further studies explored which specific features of a song may indicate quality or aggressiveness of the caller or the competitive strength of the signal. Correlational studies have shown that, for example, song rate or duration and especially song repertoire size correlate positively with health condition (e.g. parasite load or food supplemented treatments), age, attractiveness to females and competitive abilities in obtaining or maintaining territories (Becker 1982; Vehrencamp 2000; Collins 2004; Garamszegi 2005). In addition, aggressive signalling was inferred from measuring changes in male vocalisations (e.g. song rate, complexity, or song type switching) from baseline signalling to signalling when the resident bird is challenged by an intruder (Kramer *et al.* 1985; Smith & Smith 1996). However, some researchers limit the definition of aggressive signals to those that correlate with physical aggressiveness (Searcy & Beecher 2009; Akçay *et al.* 2013; Searcy *et al.* 2014). According to this logic, only signals that occur more often in individuals that escalate a fight, and specifically closer to a physical attack, would classify as aggressive signals, such as signal rate or specific soft songs in various species (Searcy *et al.* 2006; Baker *et al.* 2012; Moran *et al.* 2018). However, signal exchanges usually proceed physical interactions and are presumably even selected for to prevent physical interactions, so that the signalling and direct physical aggression are not necessarily correlated (Naguib & Mennill 2010; Bircher *et al.* 2023). To test which characteristics may signal overall strength, motivation or aggressiveness of the caller, one solution is to test how individuals respond to playbacks of varying acoustic signals (Catchpole & Slater 2008). Individuals are expected to adjust the strength of their response to the strength of the intruder, hence only strong challengers would elicit a strong response (Collins 2004; Bradbury & Vehrencamp 2011). Thus, we can infer the signal value of specific vocal characteristics by measuring which playback variants elicit a stronger vocal response (e.g. more vocalisations) and stronger threatening responses (e.g. close approaches, visual displays and attacks) (Moseley *et al.* 2013; Ansell *et al.* 2020). Even if one cannot determine the exact information content of those signals, consistently different responses by multiple individuals towards signal variants indicate which characteristics of a signal inform about the caller's quality, strength or aggressiveness (Vehrencamp 2000; Collins 2004; Naguib & Mennill 2010). A variety of song features have been associated with the strength of a signal or aggressiveness of the caller using one or multiple of the methods outlined in this paragraph.

Similar mechanisms may apply to vocalisations other than song, yet these remain largely untested. In only a few songbird species, specific non-song vocalisations were related to aggressive interactions or physical aggressiveness of the caller (Baker *et al.* 2012; Araya-Ajoy & Dingemanse 2014; Moran *et al.* 2018; Strauß *et al.* 2020). However, as mentioned in Chapter 1.2, also alarm calls are produced in agonistic interactions between conspecifics, and may play a vital role in territorial defence. This especially broadens the focus from mainly male song to the role of females in territorial defence also in the Northern hemisphere. Also females sing and in a variety of species (Odom *et al.* 2014), even duet with their partner in tropical and subtropical regions (Hall 2009), and male and female song was even found to be the ancestral state (Riebel *et al.* 2005; Odom *et al.* 2014). In the Northern hemisphere, however, females in many species do not sing or sing far less than males (Garamszegi *et al.* 2007; Catchpole & Slater 2008). Nevertheless, Eurasian magpie pairs defends the territory together and both frequently vocalise. The magpie song is very soft and unlikely serves as territorial signal to potential competitors in the area. Instead, magpie males and females are reported to produce short, harsh calls and the "chatter" call during territorial defence.

In this thesis, I therefore study if variations in the "chatter" alarm call in Eurasian magpies signal strength or aggressiveness during territorial defence, using playback experiments. Specifically, we study if coordination between a pair, i.e. the playback of overlapping chatter calls, elicits a stronger vocal and approach response of territory owners than the playback of alternating chatter calls in **Chapter 4**. In **Chapter 5**, we further test if longer chatter calls or a higher rate of chatter calls elicit a stronger response of magpie pairs than shorter chatters at a low rate. I thereby expand the field in which alarm calls are classically investigated (i.e. in predatory contexts) to test how variation in alarm calls are perceived in a territorial intrusion context.

(1.5) Summary

Alarm calls are an important model for animal communication. A variety of species use highly specialised calls when encountering particular predators, while others use general calls for a variety of threats, including conspecifics. Still, many questions remain, for example why acoustic signals are more complex in some species than in other species. Specifically, in species with a general alarm call that is used across contexts, it remains open to what extent variation in this call provides information about the caller or the context. I therefore tested a set of hypotheses on the use and function of the seemingly general "chatter" alarm call in Eurasian magpie (Figure 1.1). In this thesis, I studied:

1. The context-specificity of call variations, i.e., if variations of the chatter call are specific to certain predatory or conspecific threats and if the behavioural responses to those threats likewise vary between the threat types (**Chapter 2**).
2. Why magpies likely alarm call, through testing how the social environment alters alarm calling behaviour in magpies (**Chapter 3**).
3. If variation in chattering contains information on a territorial intruder's strength or aggressiveness by testing if a presumably higher coordination of an intruding pair, simulated through overlapping of two birds' "chatter" calls, elicit a stronger response of the territorial pair than playback of alternating chatter calls (**Chapter 4**).
4. If variation in chatter duration and rate contains information on an intruder's strength or aggressiveness by testing if playbacks of long or high rates of chatters elicit a stronger response from magpie pairs than short chatters at a low rate (**Chapter 5**).

Finally, I synthesise all findings in **Chapter 6** and discuss not only likely functions of magpie alarm calls but also their implications for future studies on alarm calling and territorial defence in birds.

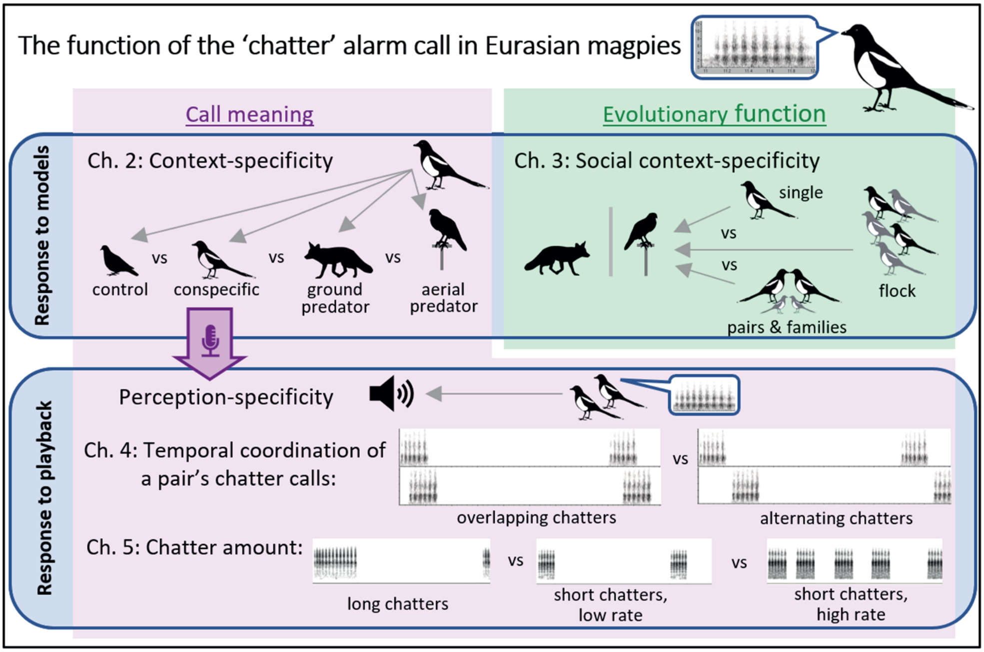

Figure 1.1. Schematic overview of this PhD thesis, in which I tested approach, threatening and vocal responses to taxidermic models representing different threat types in Chapters 2 and 3, and tested approach and vocal responses to playbacks of different chatter variations in Chapters 4 and 5.

Ch. 2 | The “chatter” call in Eurasian magpies (*Pica pica*) is an alarm, mobbing, and territorial defence signal

Miriam Kuspiel, Arnout Lindeman, Marc Naguib, Sjouke A. Kingma

(2.1) Abstract

Alarm calls are widely used in anti-predator responses and have been extensively studied for their complexity and information content. While distinct call types are often studied in the context of distinct anti-predator responses, alarm calls are also often produced in agonistic interactions with conspecifics. We therefore determined how the usage of a highly variable “chatter” alarm call in Eurasian magpies (*Pica pica*) differs between distinct predatory and territorial intrusion contexts, and also if other behaviours differ between those contexts. By placing taxidermic models of ground predators (red fox), aerial predators (Eurasian sparrowhawk), conspecifics, or controls (domestic pigeon) near magpie pairs in the wild, we reveal that chatter calls of highly variable syllable numbers are produced in great numbers towards conspecific as well as ground predator models. Likewise, magpies approached and swooped over both types of threat. In contrast, magpies neither closely approached nor chatter-called much in aerial predator and control trials. Nevertheless, those few chatters that were emitted in aerial predator trials were significantly shorter, faster, and lower in pitch than in all other trials. Taken together, chatter calls can be used as an aggressive, mobbing, and warning signal in social conflicts with conspecifics and in predator-induced contexts. This highlights that the same call type, with or without subtle variation, functions across a range of contexts and emphasizes the importance to integrate multiple facets of alarm calling when studying context specificity.

KEYWORDS: alarm call, anti-predator behaviour, bird, communication, corvid, mobbing, territorial defence.

(2.2) Introduction

Acoustic communication in animals serves a variety of functions. In agonistic interactions, for instance, vocal signalling can reduce the costs of escalating physical attacks when the signals inform about the signaller's characteristics (e.g. its strength or quality) or its internal state, such as its motivation (Bradbury & Vehrencamp 2011). Moreover, when vocalisations reflect the arousal of an individual, receivers could gather information about a conflict situation or the presence and the degree of threat of a predator (Manser 2009). Individuals may even emit certain vocalisations (e.g. alarm calls) specifically to warn others about threats (Klump & Shalter 1984; Blumstein & Armitage 1997; Griesser 2013). Calls given in such situations of high arousal are thus a prime subject to exploring complexity and meaning of acoustic signals.

Many researchers have explored the context-specificity of alarm calls. Various species use distinct alarm call structures for specific types of threats, for instance for predators attacking from the air (Macedonia & Evans 1993; Gill & Bierema 2013; Townsend & Manser 2013; Suzuki 2016). Such context-specific calls enable receivers to adopt an adaptive response such as a specific escape direction or to approach and mob the threat (Manser *et al.* 2002; Griesser 2008; Cunningham & Magrath 2017). Interestingly, however, predator-specific call types did not evolve in all species and even the alarm calls of vervet monkeys (*Chlorocebus Pygerythrus*) are less context-specific than previously thought (Price *et al.* 2015). In fact, multiple species produce general alarm calls in a variety of contexts, including conspecific interactions (Collier *et al.* 2017; Dezecache & Berthet 2018; Fichtel 2020).

The existence of general alarm calls raises the question whether specific calls are not selected for because the anti-threat response (whether flight, general alert, or attack) is similar under different forms of threat (Macedonia & Evans 1993; Fichtel & Kappeler 2002; Dezecache & Berthet 2018), including that posed by conspecific rivals. Even though conspecifics usually pose a very different threat than predators, the behaviours that both invoke (such as mobbing and aggressive displays to repel the threat) can be very similar. The function of calls in mobbing and territorial contexts may then further shift from pure warning to recruiting conspecifics and repelling the threat (Cunningham & Magrath 2017; Fichtel 2020). Thus, even if a call may not be specific to the type of threat, it could still be specific to a particular adaptive anti-threat response. To understand the use, specificity, and evolution of general alarm calls, it is therefore crucial to compare and integrate behavioural responses to different predatory and conspecific threats.

Regardless of whether or not calls are context-specific, threat-induced calling may vary gradually. As such, graded variation in call structure, rate, frequency, or combinations of calls or notes at different rates may encode information about the urgency of a threat (Naguib *et al.* 1999b; Manser 2001; Baker & Becker 2002; Blumstein 2007; Carlson *et al.* 2017). For instance, graded variation appears to be specific to the behavioural responses towards threats in black-billed magpies (*Pica hudsonia,* Stone & Trost, 1991), where a short-syllable variant of the alarm call is associated with alarm-only behaviours and retreat. The so-called basic alarm call, on the other hand, is given mostly together with mobbing the predator and elicits receivers to approach. Hence, revealing subtle variation can be essential in understanding the specificities of otherwise seemingly general alarm calls.

Here, we determined experimentally whether an apparent lack of context-specificity of the general “chatter” call of Eurasian magpies (*Pica pica*) made in response to various predatory and conspecific threats (Birkhead 1991) can be explained by similar behavioural responses to different threats. We further tested whether graded variation within the chatter calls is specific to those threatening contexts. To do so, we simultaneously investigated the behavioural (escape, approach, and attack) and acoustic (call rate, call duration, syllable duration, and frequency) responses of wild magpies towards taxidermic models of a ground predator, an aerial predator, a conspecific, and a control species. Because magpies are highly territorial against conspecifics and mob ground predators such as foxes and domestic cats (Birkhead, 1991), we expected similar behavioural responses to those threats. In contrast, we predicted that magpies respond more cautiously to aerial predators that are more mobile and could pose a stronger threat. Accordingly, we predicted that ground predators and conspecifics elicit similarly high rates of chatter calls, whereas aerial predators elicit no chatters or low chatter rates that might differ subtly in call structure.

(2.3) Methods

(2.3.1) Study system

We studied free-living magpies in Ede, Wageningen and Bennekom, the Netherlands (N52° 00.672' E5° 40.020'), from 4th June until 27th July 2022. Magpies are common in the Netherlands and occur at high densities especially in urbanised areas, with up to 24 territories per square kilometre and four to five hectare territory sizes in similar high-density populations (Birkhead 1991; Lehmann *et al.* 2005). These all-purpose territories are defended year-round and offspring stay for only several weeks on their natal territory after fledging in June/ July, before they join non-territorial flocks (Baeyens 1981b; Birkhead 1991). While the exact breeding stage of the magpies included in this study was unknown, we observed fledglings with their parents throughout the study period.

(2.3.2) Experimental setup

Experiments were conducted between 0600 and 1500 hours and when temperatures were below 25°C. We cycled through the study area scouting for magpies. Once found, we placed the experimental set-up in view of the magpies at 23.3 ± 11.2 m distance (mean ± SD) in the horizontal plane from the closest magpie, depending on the location of the magpies(e.g. on the ground or in a tree), the specific habitat structure (open or dense), and the magpies' movements during set-up. All models (see below) were hidden under a green plastic tarp during transportation and set up. To start a trial, the cover was removed with a string from at least 15 m distance from the model and closest magpie (depending on the openness of the observation area) and the behaviours and alarm calls of magpies in the subsequent ten minutes were recorded. The ten-minute observation time was sufficient to detect variation in magpie responses between trials and unlikely driven by a difference in the latency to detect the stimulus (latency to the first call was mostly within 20 seconds, data not shown). Distances between the model and main locations of magpies during the trial were measured after the trial. Since no magpies in our study area were individually marked, we reduced the probability of sampling the same individuals multiple times by ensuring that trial locations were at least 200 m apart (based on neighbour-nest distances in other European magpie populations with similarly high breeding densities; Birkhead, 1991; Lehmann et al., 2005).

Magpies were exposed to taxidermic models of an aerial predator, i.e. either one of three female Eurasian sparrowhawks (*Accipiter nisus*, n = 13 trials), a ground predator, i.e. one of two red foxes (*Vulpes vulpes*, n = 14 trials), a conspecific, i.e. one of three magpies (n = 16 trials), or a control species, i.e. one of two domestic pigeons (*Columba livia domestica*, n = 12 trials). Both sparrowhawks and foxes have been reported to predate on magpies, to be mobbed by them (Birkhead 1991; Stone & Trost 1991), and both occur commonly in our study area. Sparrowhawk taxidermic models were placed on a small platform on a 1.08 m high pole made from birch wood to simulate natural perch behaviour. Pigeon models were also placed on the pole to standardize this with the presentation of the sparrowhawks. The fox and magpie models were presented on the ground. Trial order was set by randomising threat type within sets of all four model types. We randomly assigned replicates to each threat type (ensuring even usage of all models). This set order was then adjusted on a trial day to account for limitation in the number of models that could be carried.

To score the behaviours of magpies in response to the models, we noted locations, approaches and retreats of magpies, occurrence of apparent threatening behaviour (circling over or diving near the model, hereafter referred to as swoops), and attack behaviours (hitting the model with the feet or pecking at it) during the trial onto audio recording devices. We measured the closest approach distance (from the model to the point to which any of the magpies approached in the horizontal plane) and the start distance (distance between the model and the position of the closest magpie at start in the horizontal plane). In the single case where the closest magpie flew further away from the model immediately after uncovering and did not approach again, the landing distance was taken as closest approach distance. As a measure of risk-taking, we scored if magpies approached on the ground within 15 m of the model.

Magpie vocalisations were recorded as wav files throughout the trial with a directional microphone (Sennheiser ME66/K6 with a velour foam windshield) pointed at the target area and connected to a Tascam DR100 MKIII digital audio recorder. Individual callers could not be reliably identified due to vegetation and because individuals were unmarked. To control for the number of potential callers, we noted the maximum number of magpies present during a trial (range 2-7 individuals, min. 1 pair).

We conducted 59 trials on magpie pairs with (n = 25) and without (n = 34) fledglings. For the analysis, we excluded three trials in which a cat appeared near the experimental site. Trials with only a single individual or flocks present were not included. Magpies were classified as pair if they were seen together during the trial, they did not fly away into different directions or if they stayed closer to each other than to other adult magpies (unless involved in agonistic interactions with other adult magpies). Families (pairs with fledglings) were identified based on their behaviours (staying close to or returning to each other, begging) and were included in this study. Slight differences in plumage characteristics, timing of moult, and begging behaviours allowed us to differentiate juveniles from adults while males and females were often too similar for us to differentiate reliably (Birkhead 1991).

(2.3.3) Acoustic analyses

We identified chatter calls on the sound recordings by listening to the sounds and by inspecting spectrograms in Audacity® v3.2.4 (Audacity Team, 2023, Hann window with 1024 FFT length and zero padding factor 2, linear scale, 20 dB gain and 80 dB range). By setting labels with syllable counts over all chatter calls in Audacity and exporting them with label start and end times as text file, we extracted the number and duration of all chatter calls per trial in R v4.2.2 (R Core Team 2022). Calls that were noted by the observers as distant or calls that were faint on the recordings were excluded from the analyses. Chatters with unclear duration (due to noise), that were combined with non-chatter syllables, and overlapping chatter sequences were distinguished from clear chatter calls. For overlapping chatters, we marked the duration of the entire chatter sequence and numbers of chatters that overlap, but syllable counts and durations per chatter remained unclear in these cases.

We exported all labels as text files (including start and end times of each label) and merged them with trial information in R v4.3.2 (R Core Team, 2023). We used the packages tidyr v1.2.0 (Wickham & Girlich 2022) and dplyr v1.0.8 (Wickham *et al.* 2022) to extract the total counts of all chatter calls (including unclear and overlapping chatters) per trial, the total duration of clear chatters plus overlapping chatter sequences (s) per trial, the number of syllables per clear chatter and the individual chatter call duration divided by the number of syllables of the same chatter call as estimate for syllable duration per chatter call.

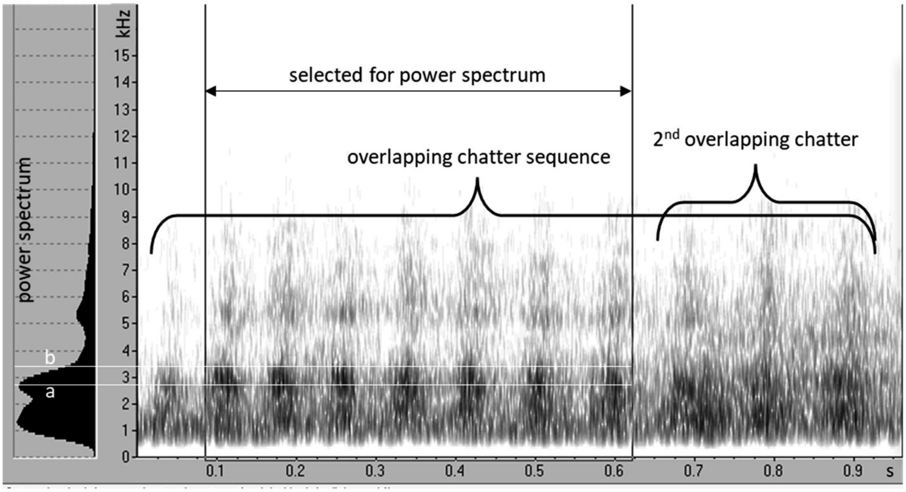

Figure 2.1. Spectrogram of overlapping Eurasian magpie alarm chatter calls and the corresponding power spectrum with amplitude against frequency (left). Marked are the entire chatter sequence, the second overlapping chatter, and the part of the first chatter selected for computing the power spectrum to determine the (a) peak frequency of 2580 Hz and (b) corresponding maximum bandwidth at 3440 Hz, -10 dB from the peak.

To measure the spectral parameters of chatter calls, we loaded the first one to five chatters of a trial (excluding distant chatters and those that were too unclear to be measured) into Avisoft-SAS Lab Pro v5.3.2-14 (Specht 2023). Calls were high-pass filtered with cut-off frequency at 800 Hz and 100 Hz transition interval to filter out loud background noise below the main chatter calls for better peak detection in chatter calls (see below). For each call, we selected the clearest and non-overlapping syllables from the spectrogram (Hamming window with FFT length 512, 25 % frame size and 93.75 % overlap), computed the logarithmic power spectrum and extracted the peak frequency as the amplitude peak of the highest frequency within the lower chatter band as well as the corresponding maximum bandwidth at -10 db (Figure 2.1). Hysteresis and threshold were adapted or peak or bandwidth was measured manually if peaks in background noise were higher than the actual chatter peak.

(2.3.4) Statistical Analyses

We used generalised linear (mixed) models in the package glmmTMB v1.1.4 (Brooks *et al.* 2017) with the appropriate family distribution in R v4.3.2 (R Core Team 2022) to test for the effects of the threat type on each of the response variables (see below), including only necessary co-variables (see below). Since the replicates of the different threat types did not explain any variation (variance <0.01), they were not included as random effect (data not shown). We included the square-root transformed distance between the model and the closest magpie at start as fixed factor in all models to test if the urgency of a threat alters the response of the magpies. The start distance did not differ between threat types (χ^2_3 = 1.86, P = 0.60). We used the Dharma package v0.4.6 (Hartig 2022) to assess model fits and violations of assumptions (e.g. overdispersion). We selected the link family function based on the type of data (i.e. binomial, continuous or counts) and on the Dharma model fit evaluations. We checked that collinearity was low (VIF < 2.5; Johnston et al., 2018) for all models with the package performance v0.10.5 (Lüdecke *et al.* 2021).

For non-vocal behaviours, we first modelled the effect of threat type on the square-root-transformed closest approach distance (distance to the model at closest approach), using a gaussian family distribution and including the square-root transformed start distance as fixed factor. We square-root transformed the distances to better reflect threatening and risk-taking behaviour where small differences at close distances are considered more threatening or risky than equally small differences at greater distances. We further modelled the effect of threat type on how much magpies approached (distance moved), calculated as the square root of the closest approach distance subtracted from the square root of the start distance, using a tweedie family distribution with a log link function and including the square-root transformed start distance as fixed factor.

For binary data on if magpies approached on the ground within 15 m and if they swooped during a trial, we tested for an effect of threat type with a two-sided fisher’s exact test. We did not use a generalised linear model here because all values for one threat type were zero (no response) and no estimates could be calculated for this threat type. Since magpies almost never attacked the model (n = 2 trials), we did not test for an effect of threat type on this behaviour.

To test whether threat type affected how much magpies chattered, we selected the negative binomial family distribution “nbinom1” with a log link function for the total number of chatter calls per trial, and a “tweedie” family distribution with a log link function for the total chatter duration of clear chatter calls and overlapping chatter sequences. In addition to the start distance, we included the number of magpies present as independent variable in both models.

To test for an effect of threat type on chatter characteristics, trial number was additionally included as random variable (since multiple calls per trial were included) in all following models. We used the “nbinom2” family distribution with a log link function for the model on the individual chatter duration, i.e., the number of syllables per chatter. For the analyses of the average syllable duration per chatter, we used a gaussian distribution and included the number of syllables of the chatter as independent variable. Minor, negligeable heterogeneity of variance, deviance from normal distribution and outliers persisted in models of the number and durations of chatter syllables. For the models on both the peak frequency and maximum bandwidth frequency, we used a gaussian family distribution and no additional variables. Density + interval (half-eye) plots were created with the ggdist package v3.2.1 (Kay 2023). We including the square-root transformed start distance as fixed factor in all models on chatter characteristics.

We used Anova Type II analysis-of-variance tables for model objects (rstatix package v0.7.0, Kassambara, 2021) to obtain test statistics for the effects of threat type and independent co-variables in all models. We subsequently computed the estimated marginal means (least-square means) with corresponding standard errors for each threat type and the pair-wise comparisons between threat types using the package emmeans v1.8.2 (Lenth 2022).

(2.3.5) Ethical Note

We followed the *Guidelines for the treatment of animals in behavioural research and teaching* (ASAB/ABS 2020) to plan the experiments with a minimum impact on the tested birds. Eurasian magpies are not endangered in the Netherlands and we did not catch them. We conducted trials (ca. 20 mins in total) in urban areas where magpies frequently encounter humans, cyclists, cars, and predators like domestic cats. We limited the trial duration and kept the models covered between trials to minimise the level of distress to the birds. We further kept a distance of minimally ten meters to adult and fifteen meters to fledgling birds not to stress them.

(2.4) Results

(2.4.1) Behavioural response to conspecifics and predators

Magpies approached models of a conspecific significantly more closely than models of a pigeon (control) or sparrowhawk (aerial predator, Table 2.1, Figure 2.2.a). In some trials, magpies closely approached fox models (ground predator) to up to 0.5 m, yet the average response to ground predators did not significantly differ from any other threat type (P = 0.08 to 0.25; Table 2.1, Figure 2.2.a). The start distance positively affected the closest start distance, i.e. magpies approached less closely in trials were the initial distance of magpies to the model was greater (Table 2.1). Magpies also significantly differed in how much they approached between threat types, with again magpies approaching over are longer distance in conspecific trials than they did in other trials (Table 2.A-1, Figure 2.A-1). In none of the trials we observed clear escape behaviour.

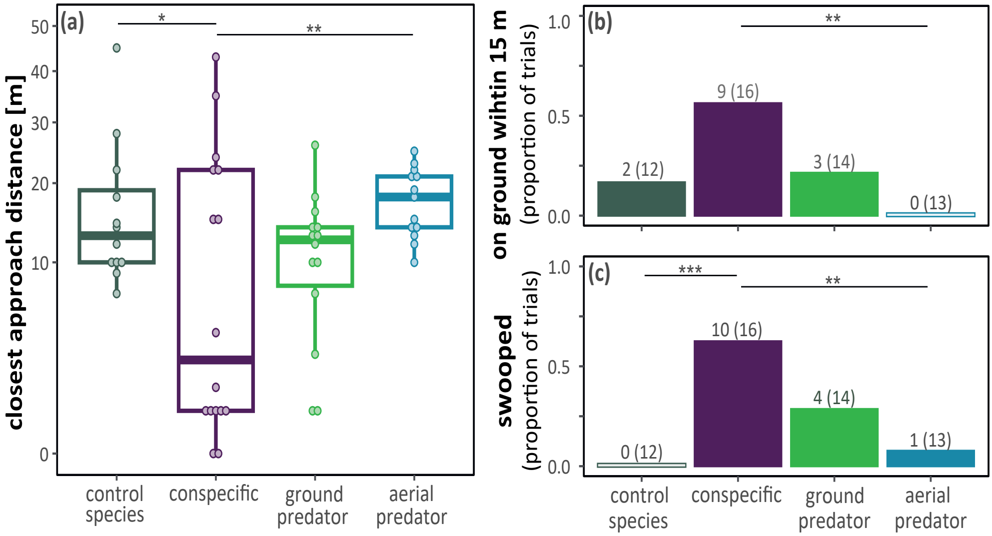

Figure 2.2. (a) The closest approach distance of magpies to taxidermic models of different threat types (x-axis) for each trial (circles) and boxplots per threat type displaying interquartile range (box), median (bold line) and the range of the data within 1.5 times the interquartile range (whiskers). (b) Proportion of trials in which magpies approached on the ground within 15 m of the model, or (c) swooped over the model of different threat types (x-axis). Sample sizes of the proportions are given as number of trials with approaches or swoops (the number of all trials). Significant pairwise differences between threat types are indicated through horizontal lines (* $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$).

Whether or not magpies approached on the ground within 15m of the model depended on what threat type was presented (two-sided Fisher's exact test: *P* = 0.004). Magpies were more likely to get close to a conspecific model than an aerial predator model, while all other threat type comparisons were not significant (Table 2.2, Figure 2.2.b). Magpies also differed significantly across threat types in the proportion of trials in which they threatened the model (two-sided Fisher's exact test, *P* = 0.0004) with magpies swooping over models in significantly more conspecific trials than control and aerial predator trials (Table 2.2, Figure 2.2.c). Magpies physically attacked the model in only two trials with a model of a conspecific and in no other trial.

Table 2.1. Post-hoc pair-wise comparisons between threat types of the closest approach distance of magpies to the model. Significant pair-wise comparisons are printed in bold. Test statistics of all model variables and sample sizes are given below the comparisons.

Threat comparisons	**estimate (SE)**	t_{49}	***P***
control species - conspecific	1.33 (0.54)	2.46	**0.018**
control species - ground predator	0.65 (0.56)	1.16	0.25
control species - aerial predator	-0.32 (0.57)	-0.57	0.57
conspecific - ground predator	-0.67 (0.52)	-1.30	0.20
conspecific - aerial predator	-1.65 (0.53)	-3.12	**0.003**
ground predator - aerial predator	-0.97 (0.54)	-1.79	0.080
Statistics of the main model:			
threat type	X^2_3 = 11.54, ***P* = 0.009**		
sqrt(start distance)	estimate (SE) = 0.41 ± 0.19, X^2_1 = 4.34, ***P* = 0.037**		
sample sizes (trials)	12 (control), 16 (conspecific), 14 (ground predator), 13 (aerial predator)		

Table 2.2. Pair-wise fisher-exact tests of threat type comparisons of the proportion of trials in which Eurasian magpies approached on the ground within 15 m (risk-taking) or swooped over the taxidermic model (threatening). Significant pair-wise comparisons are printed in bold.

Threat comparisons	**N**	***P* (risk-taking)**	***P* (threatening)**
control - conspecific	28	0.054	**0.0009**
control - ground predator	26	1.00	0.10
control - aerial predator	25	0.22	1.00
conspecific - ground predator	30	0.072	0.081
conspecific - aerial predator	29	**0.001**	**0.006**
ground - aerial predator	27	0.22	0.33

(2.4.2) Acoustic responses to conspecifics and predators

Magpies significantly varied in the number of chatters depending on the threat type (Table 2.3.a, Figure 2.3). Magpies rarely chattered in control and aerial predator trials, and seven to 17 times more often in conspecific trials or ground predator trials than in control trials and aerial predator trials (Table 2.3.a, Figure 2.3). Magpies chattered significantly more if more magpies were present, but less if the model was placed at a greater distance from the magpies at start (Table 2.3.a). Also for the total duration of all clear chatters and overlapping chatter sequences, we found that magpies rarely chattered in control and aerial predator trials but substantially more in conspecific trials and ground predator trials (Table 2.A-2.a, Figure 2.A-2).

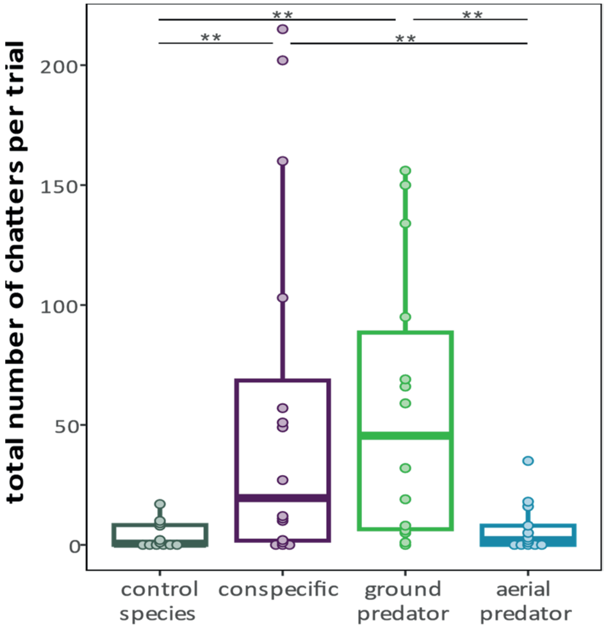

Figure 2.3. Total number of chatters in a trial (circles) where magpies were exposed to taxidermic models of different threat types (x-axis), with boxplots per threat type displaying interquartile range (box), median (bold horizontal line) and the range of the data within 1.5 times the interquartile range (whiskers). Significant pair-wise differences are indicated through horizontal lines (** *P* < 0.01).

Table 2.3. Post-hoc pair-wise comparisons between threat types of magpie chatter numbers and characteristics. Significant pairwise comparisons are printed in bold. Test statistics of all model variables and sample sizes are given below the comparisons.

	(a) Total number of chatters			**(b) Number of syllables per chatter**			**(c) Syllable duration per chatter**			**(d) Peak frequency per chatter**		
Threat comparisons	**estimate (SE)**	***z* ratio**	***P***	**estimate (SE)**	***z* ratio**	***P***	**estimate (SE)**	**t_{1113}**	***P***	**estimate (SE)**	**t_{129}**	***P***
control - conspecific	-1.42 (0.46)	-3.05	**0.002**	-0.26 (0.19)	-1.33	0.18	0.002 (0.06)	0.60	0.65	44.43 (78.42)	0.57	0.57
control - ground predator	-1.28 (0.46)	-2.78	**0.005**	-0.22 (0.19)	-1.16	0.25	-0.003 (0.06)	-0.48	0.57	45.17 (76.67)	0.59	0.56
control - aerial predator	-0.02 (0.53)	-0.03	0.97	0.22 (0.22)	1.02	0.31	0.01 (0.06)	2.51	**0.019**	223.86 (99.50)	2.25	**0.026**
conspecific - ground predator	0.14 (0.33)	0.41	0.68	0.04 (0.12)	0.29	0.77	-0.01 (0.04)	-1.60	0.12	0.74 (58.72)	0.01	0.99
conspecific - aerial predator	1.40 (0.43)	3.27	**0.001**	0.48 (0.16)	3.06	**0.002**	0.01 (0.05)	2.65	**0.009**	179.43 (87.48)	2.05	**0.042**
ground - aerial predator	1.27 (0.42)	3.04	**0.002**	0.44 (0.16)	2.84	**0.005**	0.02 (0.05)	3.97	**0.0001**	178.70 (85.45)	2.09	**0.038**
Statistics of the model:												
threat type	$\chi^2_3 = 18.31$, **$P < 0.001$**			$\chi^2_3 = 10.65$, **$P = 0.011$**			$\chi^2_3 = 16.21$, **$P = 0.001$**			$\chi^2_3 = 5.83$, $P = 0.12$		
number of magpies	estimate (SE) = 0.35 (0.12), $\chi^2_1 = 14.08$, **$P < 0.001$**			-			-			-		
sqrt(start distance)	estimate (SE) = -0.57 (0.15), $\chi^2_1 = 7.84$, **$P = 0.005$**			estimate (SE) = 0.05 (0.04), $\chi^2_1 = 1.30$, $P = 0.25$			estimate (SE) = -0.001 (0.001), $\chi^2_1 = 0.72$, $P = 0.40$			estimate (SE) = 33.80 (24.70), $\chi^2_1 = 1.87$, $P = 0.17$		
number of syllables	-			-			estimate (SE) = 0.003 (0.0003), $\chi^2_1 = 66.87$, **$P < 0.001$**			-		
trial (random effect)	-			variance (STD): 0.07 (0.27) (1121 observations, 37 trials)			variance ± STD: 0.0001 (0.01) residual: 0.0001 (0.01) (1121 observations, 37 trials)			variance ± STD: 12,748 (112.9) residual: 30,368 (174.3) (136 observations, 33 trials)		
sample sizes (trials or chatters/trials)	12 (control), 16 (conspecific), 14 (ground predator), 13 (aerial predator)			36/4 (control), 437/13 (conspecific), 572/13 (ground predator), 76/7 (aerial predator)			36/4 (control), 437/13 (conspecific), 572/13 (ground predator), 76/7 (aerial predator)			19/5 (control), 49/11 (conspecific), 56/12 (ground predator), 12/5 (aerial predator)		

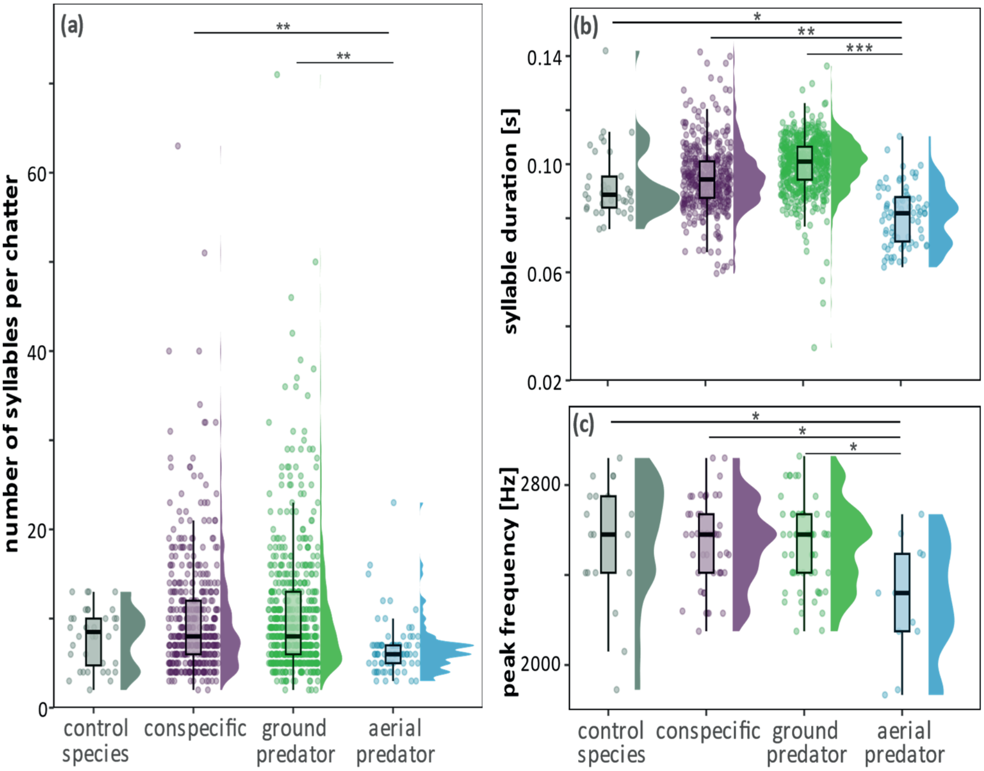

Figure 2.4. (a) Number of syllables, (b) syllable duration, and (c) peak frequency of the lower chatter band of chatter calls magpies made in response to models of the different threat types (x-axis). Shown are the datapoints of all measured chatters (circles), boxplots displaying interquartile range (box), median (bold horizontal line) and the range of the data within 1.5 times the interquartile range (whiskers), and density + interval (half-eye) plots per threat type. For graphical purpose, plot (a) excludes one datapoint of a 124-syllable chatter in a conspecific trial. Significant differences of the model comparisons are indicated through horizontal lines (* $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$).

Magpies emitted chatters of three to 124 syllables (Figure 2.4.a) and significantly varied in the number of syllables per chatters depending on threat type (Table 2.3.b). While shorter chatters of five to seven syllables were emitted in trials with all model types, longer chatters were mainly emitted in conspecific and ground predator trials (Figure 2.4.a). Thus, the mean number of syllables per chatter was significantly lower in aerial predator trials than in ground predator and conspecific trials (Table 2.3.b). In addition, magpies significantly varied in the duration of chatter syllables between threat types and called significantly faster when presented with an aerial predator model than in control, conspecific and ground predator trials (Table 2.3.c, Figure 2.4.b). Similarly, magpies in aerial predator trials chattered with a significantly lower peak frequency (of the lower chatter band) than magpies in control, ground predator and conspecific trials (Table 2.3.d, Figure 2.4.c), although the overall effect of threat type on peak frequency was marginally non-significant (*P* = 0.053; Table 2.3.d). The maximum frequency bandwidth at -10 dB from the measured peak did not differ among the treatments (Table 2.A-2.b, Figure 2.A-3). The variation in the start distance between trials did not significantly affect any of the characteristics of single chatter calls (Table 2.3.b to d).

(2.5) Discussion

Our study reveals that magpies varied their behavioural and vocal response to different threat types. Magpies closely approached and threatened (swooped over) models of a conspecific significantly more than models of a sparrowhawk (aerial predator) and pigeon (control). Responses to a fox model (ground predator) were intermediate and did not significantly differ from responses to any other model type. In terms of vocal responses, magpies chattered significantly more towards both conspecific and ground predator models than towards control and aerial predator models. Moreover, chatters were on average shorter and lower in pitch when magpies were confronted with an aerial predator. These results indicate that magpies behaved and called similarly to conspecifics and ground predators whereas responses to aerial predators were mostly distinct in behavioural measures and chatter call characteristics. Thus, the chatter call differs from typical anti-predator alarm call systems since it is used with subtle variation across territorial conflicts and different types of predator defence, as discussed below.

Our findings that magpies behaved and called similarly to conspecifics and ground predators is in line with our prediction. The close approach to a conspecific and swoops and attacks in combination with vocalisations are typical territorial defence behaviours in birds (Baeyens 1981b; Akçay *et al.* 2013; Araya-Ajoy & Dingemanse 2014; Naguib *et al.* 2022; Walters *et al.* 2023). Moreover, these same behaviours also reflect an anti-predator mobbing response when expressed towards a ground predator (Curio 1978; Koboroff *et al.* 2013; da Cunha *et al.* 2017a; Carlson & Griesser 2022). In both cases, the urgency to respond is high, yet the stake and risk for the attacking individual differ. Repelling conspecific intruders is essential for maintaining a territory and future reproduction and often poses no immediate live threat (Krebs 1982; Birkhead 1991; Stamps & Krishnan 1995), while approaching a predator may increase own or especially offspring's survival but can be highly dangerous (Caro 2005; Clark 2005; Crofoot 2013; Teunissen *et al.* 2020). This difference could explain why magpies closely approached and swooped in only some ground predator but the majority of conspecific trials. Still, magpies chatter-called frequently in both ground predator and conspecific trials. We hypothesise that the function of the loud and harsh chatter call in both contexts is to harass and repel the opponent (Klump & Shalter 1984; Ficken & Popp 1996; Baker *et al.* 2012), while conspecific receivers can obtain specific information on the threat type from the context they observe (Wheeler & Fischer 2012; Fichtel 2020).

Magpies varied greatly in the duration of chatters towards both conspecifics and ground predators within and across trials. High call rates and long chatters may be associated with great arousal or aggressiveness of the caller, or a greater motivation to chase the opponent out of the territory (Szymkowiak & Kuczyński 2017). Multiple factors, such as the distance of the threat from the callers or the core of their territory, may alter the urgency and arousal of the caller and resulting call characteristics (Baker & Becker 2002; Yorzinski & Vehrencamp 2009; Ansell *et al.* 2020). Our experiments pointed in that direction since a shorter distance between model and magpies at start increased the number of chatters in a trial. This remains to be tested directly by systematically varying the start distance between trials for each threat type. Furthermore, arousal might also increase with the persistence of the threat that does not react to the harassment (Searcy & Beecher 2009) and explain variation within trials. Very loud, long calls may further require higher energy (Gil & Gahr 2002) and thus indicate the strength of the caller towards a conspecific intruder (Snijders *et al.* 2017). Such differences between callers could contribute to the chatter variation both between and within a trial. In addition, shorter calls might be interspersed with long calls to reduce the costs of calling, or to alert conspecifics of the presence of a threat, as discussed below. Graded variation within this call is thus likely linked to both the arousal of and costs for the caller, yet the underlying mechanisms remain to be explored.

Magpies did not show mobbing behaviours towards the aerial predator or control model and also chatter-called less in those trials, presumably for different reasons. They may have stayed away from the aerial predator to lower the risk of being predated and stayed away from the control because it posed no threat at all. Nevertheless, magpies produced significantly shorter and lower-pitched chatters in aerial predator trials, indicating that they did discriminate between contexts. In superb fairy-wrens (*Malurus cyaneus*), subtle differences in call structures in context of aerial predators compared to predator mobbing contexts elicit a more fearful response by conspecifics (Tegtman & Magrath 2020). Similarly, magpies appear to warn about a dangerous threat with a chatter variant while avoiding other, risky, behaviours. Warning call structures are hypothesised to evolve to reduce the risk of the caller being detected by the predator and should thus be short and produced at low rates (Klump & Shalter 1984; Caro 2005). The pitch of alarm whistles in songbirds is even beyond hearing range of raptors to avoid detection (Klump & Shalter 1984). While we did not observe such whistles in magpies, their chatter calls produced in the presumed high-threat context are short (i.e. contain fewer and shorter syllables) and are produced much more sparingly than chatter calls in the other contexts, hence probably reducing the risk of being detected by the predator. Also black-billed magpies, closely related to Eurasian magpies, produce faster yet higher-pitched calls towards dangerous predators (raptors in the sky; Buitron 1983) and do not mob predators when emitting those calls (Stone & Trost 1991). Receivers of such shorter, lower pitch signals could thus gain information of a high threat being present. Since these call structure variations are gradual and overlap with conspecific and ground predator contexts, it does not seem that they directly inform about the specific type of the threat. Still, they can function to alert receivers of some threat being present, who can integrate information from the call structure with other, e.g. visual, cues (Wheeler & Fischer 2012). The short chatter calls produced especially in high-danger contexts thus fulfil criteria for both risk reduction of the caller and a general "high-alert" signal for receivers.

(2.5.1) Conclusion

Eurasian magpies harass conspecific intruders with similar behaviours and high chatter call rates as they do for ground predators. Chatter calls appear to be used as "mobbing" calls when magpies approached ground predators as well as conspecific intruders to repel the opponent, while a short variant was emitted supposedly as warning signal in aerial predator contexts. Thus, this study confirms the need to consider the required behavioural responses towards different types of threats as well as variation within a general alarm call when exploring its function. As such, aggression towards conspecifics should be integrated into the study of alarm calls and anti-threat responses to elucidate the meaning of alarm calls. Even a seemingly invariable system with a general alarm call for various kinds of threats can entail great complexity that must not be overlooked.

(2.6) Acknowledgements

We thank Rita Fragueira for assisting with the acoustic analysis of chatter characteristics. We also thank Kat Bebbington for advice during various stages of the project.

(2.7) Appendix

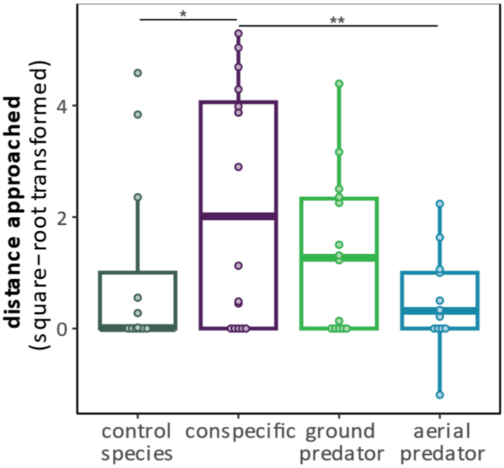

Figure 2.A-1. The distance that magpies moved closer to the taxidermic models of different threat types (x-axis) for each trial (circles) and boxplots per threat type displaying interquartile range (box), median (bold line) and the range of the data within 1.5 times the interquartile range (whiskers). Significant pairwise differences between threat types are indicated through horizontal lines (* $P < 0.05$, ** $P < 0.01$).

Table 2.A-1. Post-hoc pair-wise comparisons between threat types of the distance that magpies moved closer to the model. Significant pair-wise comparisons are printed in bold. Test statistics of all model variables and sample sizes are given below the comparisons.

Threat comparisons	**estimate (SE)**	t_{48}	***P***
control – conspecific	-1.36 (0.52)	-2.61	**0.012**
control - ground predator	-0.98 (0.57)	-1.71	0.093
control - aerial predator	0.11 (0.63)	0.18	0.86
conspecific - ground predator	0.38 (0.42)	0.90	0.37
conspecific - aerial predator	1.47 (0.52)	2.83	**0.007**
ground predator - aerial predator	1.09 (0.56)	1.95	0.057
Statistics of the main model:			
threat type	$\chi^2_3 = 11.82$, $\mathbf{P = 0.008}$		
sqrt(start distance)	estimate (SE) = 0.62 (0.18), $\chi^2_1 = 11.76$, $\mathbf{P = 0.0006}$		
sample sizes (trials)	12 (control), 16 (conspecific), 14 (ground predator), 13 (aerial predator)		

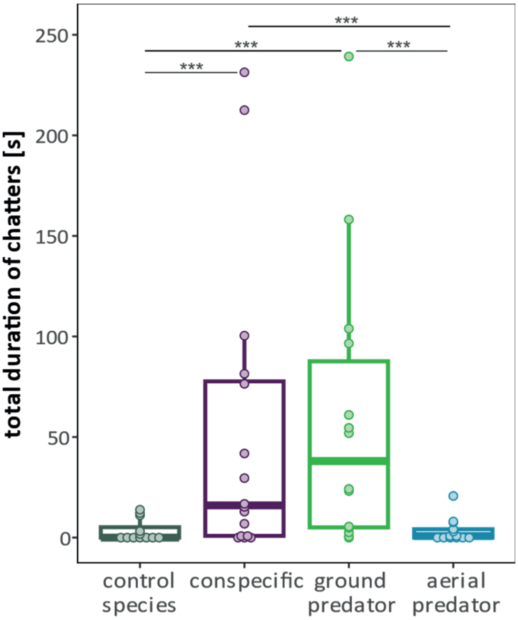

Figure 2.A-2. Total duration of single chatters and overlapping chatter sequences per trial (circles) of magpies exposed to taxidermic models of different threat types (x-axis) and boxplots per threat type, displaying interquartile range (box), median (bold line) and the range of the data within 1.5 times the interquartile range (whiskers). Significant differences of pair-wise comparisons are indicated through horizontal lines (*** *P* < 0.001).

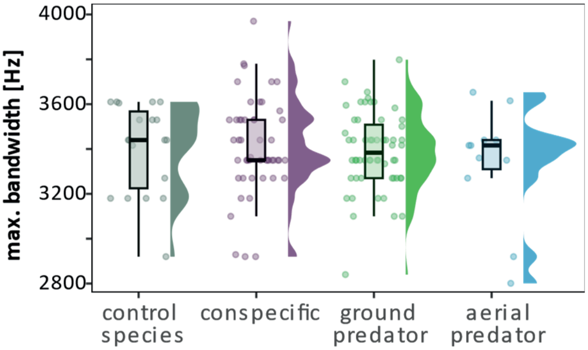

Figure 2.A-3. Frequency of the maximum bandwidth at -10 dB from the peak frequency for up to the first five magpie chatter calls per trial in response to taxidermic models of different threat types (x-axis). Shown are the datapoints of all measured chatters (circles), boxplots per threat type displaying interquartile range (box), median (bold line) and the range of the data within 1.5 times the interquartile range (whiskers), and density + interval (half-eye) plots per threat. There are no significant differences between any threat types.

Table 2.A-2. Post-hoc pair-wise comparisons between threat types of a) the total duration of all magpie chatters and overlapping chatter sequences per trial and b) the frequency of the maximum bandwidth at -10 dB from the peak frequency amplitude for up to the first 5 chatters per trial. Significant pair-wise comparisons are printed in bold. Test statistics of all model variables and sample sizes are given below the comparisons.

	a) Total duration of chattering			**b) Maximum bandwidth**		
Threat comparisons	**estimate (SE)**	***z ratio***	***P***	**estimate (SE)**	t_{129}	***P***
control - conspecific	-2.90 (0.67)	-4.30	**< 0.001**	5.54 (78.97)	0.08	0.94
control - ground predator	-2.92 (0.67)	-4.33	**< 0.001**	15.11 (77.28)	0.20	0.85
control - aerial predator	-0.15 (0.81)	-0.18	0.86	28.39 (99.21)	0.50	0.78
conspecific - ground predator	-0.02 (0.45)	-0.04	0.97	9.56 (59.47)	0.15	0.87
conspecific - aerial predator	2.75 (0.64)	4.30	**< 0.001**	22.85 (87.02)	0.50	0.79
ground - aerial predator	2.77 (0.62)	4.44	**< 0.001**	13.29 (85.02)	0.41	0.88
Statistics of main model:						
threat type	$\chi^2_3 = 36.62$, ***P* < 0.001**			$\chi^2_3 = 0.31$, $P = 0.99$		
number of magpies	estimate (SE) = 0.13 (0.20), $\chi^2_1 = 0.71$, ***P* = 0.039**			-		
sqrt(start distance)	estimate (SE) = -0.49 (0.24), $\chi^2_1 = 0.71$, $P = 0.52$			estimate (SE) = -2.62 (24.78), $\chi^2_1 = 0.01$, $P = 0.92$		
trial (random effect)	-			variance (STD): 14,319 (119.7) residual (STD): 25,535 (159.8) (136 observations, 33 trials)		
sample sizes (trials or chatters/trials)	12 (control), 16 (conspecific), 14 (ground predator), 13 (aerial predator)			19/5 (control), 49/11 (conspecific), 56/12 (ground predator), 12/4 (aerial predator)		


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Ch. 3 | Who is listening? The function of anti-predator alarm calling in Eurasian magpies

Miriam Kuspiel, Kat L. Bebbington, Marc Naguib, Sjouke A. Kingma

(3.1) Abstract

Many animals alarm call when encountering a dangerous predator. Since this conspicuous behaviour is potentially risky and costly, many hypotheses about different functions and adaptive benefits have been proposed to explain its evolution. To make inferences about the functions and benefits of alarm calling, one can assess how responses to predators vary according to differences in social settings and predator types, while taking into account the behaviour of conspecifics in response to such calls. Here, we developed a set of predictions based on these aspects to assess the function and potential benefits of alarm calling in Eurasian magpies (*Pica pica*). We exposed wild magpies in different social settings (alone, in flocks or in pairs) to taxidermic models of ground or aerial predators. We did not find support for selfish pursuit-deterrent alarm calling, since magpies rarely called in response to predators when they were alone. Equally, alarm call rates by magpies in flocks, containing potential partners, kin and co-operators, were relatively low. Neither did we observe intense communal mobbing responses in flocks. Together, these results suggest that sexual selection, kin selection and immediate mutualistic benefits (help in defence) are not the most important drivers of alarm calling in this species. Instead, alarm call rates were highest, and approach distances to predator models closest, when magpies were with their partner, during and sometimes also outside the breeding season, and regardless of whether fledglings were present. This suggests that magpies warn or protect their breeding partner or offspring when engaging in anti-predator behaviour. Consequently, we suggest that the benefits of safe-guarding partnership and reproduction is the main selection pressure for alarm calling in this, and perhaps other, year-round territorial species.

KEYWORDS: communication, alarm call, mobbing, audience effect, corvid.

(3.2) Introduction

Alarm calls are a prevalent and conspicuous element of anti-predator responses and acoustic communication across a wide range of animal taxa (Caro 2005). The evolution of such alarm calls is a fascinating topic of research, since the behaviour is presumably costly to the individual who performs it (Caro 2005; Zuberbühler 2009). In addition to opportunity costs, most notably, callers draw the attention of dangerous predators towards themselves, especially when high rates of calls are emitted and predators are approached as part of a mobbing response (Sherman 1977; Sordahl 1990; Motta-Junior 2007; Carlson & Griesser 2022). Despite such potential risk, however, alarm calling is common in many animal species, implying that there are strong benefits associated with the behaviour.

A variety of hypotheses have been proposed regarding the immediate function and the adaptive benefits that can drive the evolution of alarm calling (Zuberbühler 2009; Fichtel & Manser 2010; Carlson & Griesser 2022; summarised in Table 3.1 and detailed below). Previous studies have highlighted that assessing variation in the context under which individuals do or do not alarm call might help to unravel the evolutionary drivers selecting for the behaviour (Sherman 1977; Blumstein *et al.* 1997; Griesser & Ekman 2004; Wheeler 2008). As such, there are three important contextual components to consider. Firstly, different types of predators might pose different degrees of risk or have different ways of attacking their prey (Macedonia & Evans 1993; Randall *et al.* 2000); for birds, for instance, agile aerial predators are relatively dangerous compared to predators moving on the ground due to different escape opportunities (Hedenström & Rosén 2001). However, ambush predators (on the ground or the air) also rely on surprise effects to capture their prey (Caro 2005). Hence, alarm calls by prey to such predators might function as signal that it was detected and to demotivate the predator to attack (Caro 1995, 2005). Second, both the function and the adaptive benefits of alarm calls may be shaped by the social environment of the caller (e.g., if individuals are alone, with a partner, with offspring or with group members) (Marler & Evans 1996; Zuberbühler 2009). Other individuals offer, for example, immediate or future help, might be related to the caller, or might be reproductive partners. Finally, one can infer the function or potential benefits of alarm calling by assessing the behaviour of the caller or the receivers of the call (Gyger 1990; Evans *et al.* 1993; Zuberbühler 2009). For example, an alarm calling can function to alert conspecifics and induces joint escape (to confuse predators) or mobbing behaviour (Hamilton 1971; Curio 1978; Cresswell 1994b; Lehtonen & Jaatinen 2016). Together, therefore, differences in predator characteristics and the social environment in which alarm calling occurs, along with the associated behaviour of callers and receivers, are useful in assessing which hypotheses may explain antipredator behaviours, which is the aim of our study.

Various studies have used variation in predator type and social environment to explore the drivers of alarm calling behaviours (Sherman 1977; Blumstein *et al.* 1997; Griesser & Ekman 2004; Wheeler 2008). However, unless there is sufficient variation in social structure within a population, it can be difficult to identify or disentangle specific functions and benefits of alarm calling. Therefore, in this experimental study, we investigate alarm calling behaviour in Eurasian magpies (*Pica pica*), a species where individuals can live in different social environments. Magpie breeding pairs defend a territory throughout the year and often stay together for multiple years, while non-breeders without a territory usually roam alone or aggregate in flocks that may contain kin or a future partner (Birkhead 1991). The birds emit conspicuous chatter alarm calls ('chatters' hereafter) when encountering predators on the ground or in the air (Birkhead, 1991; Chapter 2). We presented magpies with models of mammalian ground predators and aerial predators (raptors) and specifically determined how the type of predator and the social environment (i.e. a single magpie, flock of magpies, or magpie pair with or without fledglings, during or outside the breeding season) shaped the likelihood and intensity of alarm calling and mobbing responses. We used these experiments to test a suite of predictions arising from various hypotheses related to the evolution of alarm calling, as summarised in Table 3.1.

First, one proposed function of alarm calling is to signal at predators, thereby demotivating attacks from those predators or encouraging them to leave (described as 'perception' and 'quality advertisement', or 'pursuit deterrence' hypotheses; Caro 1995; Caro *et al.* 1995; Huang & Caro 2023). Alarm calling may, in that case, have evolved because it benefits others (see below), but perhaps more importantly, because it serves an immediate selfish purpose of increased own survival. If the latter is the case, we expect individuals to respond strongly to predators regardless of the social setting (Wheeler 2008; Zuberbühler 2009), and we speculate that magpies might respond stronger to aerial predators to signal detection than to ground predators that they can avoid by staying off the ground.

Second, alarm calls could yield immediate mutualistic benefits if they function as signal to conspecifics who will subsequently immediately help in predator defence (Hypothesis 2 in Table 3.1). This help can be passive, for example if multiple individuals call or flee, thereby confusing the predator or diluting the risk for the individual (Hamilton 1971; Charnov & Krebs 1975; Sherman 1985; Cresswell 1994b; Lehtonen & Jaatinen 2016). This help can also be active, for example through shared vigilance or cooperative mobbing (Curio 1978; Clutton-Brock *et al.* 1999; Wright *et al.* 2001; Gursky 2006; Krams *et al.* 2006; Krama *et al.* 2012; Kern & Radford 2013). If such immediate mutualistic benefits indeed underlie the evolution of alarm calling, we can expect that individuals mainly call when others are in the vicinity, regardless of the relationship between caller and receivers. The predicted response to different predators then likely depends on which of these mechanisms is at play; joint predator confusion is perhaps important regardless of predator type, whereas joint mobbing might be restricted to relatively less dangerous predators (Wheeler 2008; Cunningham & Magrath 2017). In any case, if immediate mutualistic benefits are at play, we expect alarm calls to result in clear communal responses of groups, such as joint escape or mobbing, or cacophonies of alarm calls.

Third, emitting warning signals or deterring a predator may save conspecifics that are beneficial to the caller (Hypothesis 3 in Table 3.1). If so, we can expect individuals to warn and protect (3.1) group members to secure delayed mutualistic group benefits such as communal roosting in stable aggregations of familiar individuals (Smith 1986; Teunissen *et al.* 2020), (3.2) related group members to obtain indirect fitness benefits (Hamilton 1964; Maynard Smith 1965), or (3.3) their partner or offspring to improve their reproductive success (Randall *et al.* 2000; Blumstein 2007; Papworth *et al.* 2008; Magrath *et al.* 2010). Under this hypothesis, we therefore predict that alarm calls should be given in presence of group members, kin, offspring, or a breeding partner, and should take place in encounters with any predators.

Finally, future reproductive benefits may arise if opposite-sex conspecifics perceive alarm calls or predator mobbing as advertisement of a caller's quality (Wilson *et al.* 2008; da Cunha *et al.* 2017b) (Hypothesis 4 in Table 3.1). If alarm calls are indeed shaped by sexual selection, individuals are predicted to alarm call and mob more in the presence of individuals of the opposite sex, perhaps especially (but not limited to) before gaining a breeding position, and particularly when facing more dangerous predators.

From the above, it is clear that each of the different proposed hypotheses yields rather specific predictions under what social setting alarm calling should take place (Table 3.1) and how conspecifics hearing the calls should respond. We therefore measured the probability and intensity of chatter alarm calls and mobbing behaviour when magpies are presented with taxidermic models of either ground or aerial predators in relation to individuals' social environment.

Table 3.1. Overview of the hypothesised functions of alarm calling. To assess which of these hypotheses explains alarm calling by Eurasian magpies, we tested a specific set of predictions about how strongly (indicated by 0 for no response, + for a relatively weak response and ++ for a strong response) individuals should respond to predators based on their social environment and the type of predator (aerial or ground predators).

Concept	**Explanation**	**Predicted stronger response when:**			**Predicted response to different predators**	**Support from this study**
		Single	**Pair & Family**	**Flock**		
1. Own survival benefits	Signal detection or quality at (ambush) predator to deter a pursuit	+	+	+	Respond stronger to more dangerous aerial predators	No: we rarely observed single magpies to alarm call (Figure 3.1a, 3.1b), and magpies did not approach or alarm call to aerial predators more than to ground predators (Figure 3.2)
2. Immediate mutualism	Elicit others to jointly escape (confusing predator), share vigilance, or to jointly mob predator	0/+*	+	++	No difference (joint escape, vigilance) or respond stronger to ground predators (joint mobbing)	Not the primary purpose: some individuals in flocks alarm called but there was no strong immediate escape or mobbing response; overall responses were much stronger in pairs (Figure 3.1)
3. Save conspecifics						
3.1 Delayed mutualism	Warn or protect group members for (future) group-related benefits	0	+	++	Respond to any danger, or stronger to more dangerous predators	Not the primary purpose: some individuals in flocks alarm called but the intensity of calling and mobbing was much stronger in pairs (Figure 3.1b, 3.1c)
3.2. Kin selection	Warn or protect non-descendent kin	0	0	++	Respond to any danger	Maybe: we observed some calls in flocks that may contain kin**
3.3 Reproductive benefits	Warn or protect offspring or breeding partner	0	++	0	Respond to any danger, especially (i) in the breeding season or (ii) mainly in presence of fledglings	Strongly supported: intense calling and mobbing in pairs and families (Figure 3.1a-c). Responses were (i) particularly strong during the breeding season (Figure 3.2a-c) but (ii) regardless of whether or not fledglings were present (Figure 3.2d-f)
4. Sexual selection or status signalling	Advertise willingness and ability to take risks to (potential) partners	0	+	++	Respond stronger in presence of more dangerous predators	Unlikely: no high-risk mobbing in flocks (Figure 3.1b, 3.1c)

* If joined mobbing is the mechanism, single magpies may call to recruit distant conspecifics to help

** See Birkhead (1991)

(3.3) Methods

(3.3.1) Study site

In 2021 and 2022, we conducted trials on wild magpies in and around the towns of Ede, Wageningen, Renkum and Veenendaal (N51° 59.597' E5° 37.988') in the Netherlands. Magpies breed at high densities on all-purpose, year-round, territories especially in those urban areas (Birkhead 1991; Lehmann *et al.* 2005), but we also observed single magpies and aggregations of magpies (usually with magpies leaving from and arriving at trial sites independently) in our study area. Magpie fledglings remain on their natal territory for a few weeks before joining non-breeding, non-territorial flocks, until they gain a breeding territory in a later year (Birkhead 1991). Trials were conducted in the non-breeding season (October – December, after offspring have left their natal territories) and during the breeding season (June-August, when most offspring are still in the nest or living with their parents on the natal territory (Birkhead 1991). In the non-breeding season, we conducted trials earliest at dusk and latest at dawn at any temperature. During the breeding season, we conducted trials only between 06h50 and 14h30 and before temperatures exceeded 28 °C as activity was expected to drop later in the day or at higher temperatures (Verbeek 1972; Robbins 1981). No magpies in our study area were individually marked, so we reduced the probability of sampling the same individuals multiple times by ensuring that trial locations within each season were at least 200 m apart (based on neighbour-nest distances in European magpie studies with high breeding densities (Birkhead, 1991; Lehmann et al., 2005) and the perceived territory densities at trial locations).

(3.3.2) Experimental set-up

We exposed magpies to taxidermic models of ground predators (one of two models of red fox, *Vulpus vulpes*, or two models of Eurasian polecats, *Mustela putorius*, placed on the ground) or aerial predators placed on a small platform on a 1.08 m high wooden pole to mimic natural perching behaviour (one of three models of Eurasian sparrowhawks, *Accipiter nisus*). Since there was no difference between fox and pole-cat trials in the first trial period (data not shown), we therefore combined the data of both species in the analyses and only used fox models in the second trial period. Trial order was set by randomising predator type within sets of all model types. We randomly assigned replicates to each model type. This set order was then adjusted to account for limitation in the number of models that could be carried in a day and to ensure that a similar number of trials was conducted for combination of model replicate, predator type and social environment category.

We set up the predator model, covered with a green plastic tarp, when and where we encountered magpies so they could have a clear view on the model. To start a trial, we uncovered the model by pulling on a rope from a distance (> 15 m from the model and closest magpie). For the subsequent ten minutes, we recorded magpie vocalisations in wav files using a directional microphone (Sennheiser ME66/K6 with a velour foam windshield) attached to a TASCAM DR100 MKIII digital audio recorder. We further noted the number of magpies present, their start and closest locations on other recording devices. The ten-minute observation time was sufficient to detect variation in magpie responses between trials and unlikely driven by a difference in the latency to detect the stimulus (latency to the first call was mostly within 20 seconds, data not shown).

After the trial, we measured distances between the model and the closest location of any magpie at trial start and the point of the closest approach to the model of any magpie in a trial in the horizontal plane. Due to space limitations in urban environments and along agricultural fields regarding suitable locations where the model can be placed, variation in the openness of the environment, and free movements of magpies while we were setting up the model, distances between the focal birds and the predator models varied between trials (mean ± SD = 23.02 ± 11.64 m in the horizontal plane) but did not differ between threat types and social environments (Table 3.A-1). We square-root transformed the start distance and closest approach distance to reflect the greater biological importance that differences at close distances (e.g. one versus ten meters) have than differences at further distances (e.g. 20 versus 30 meters). If a magpie flew away within the first minute of the trial and no magpie approached closer anymore (n = 4 trials), we used the location it flew to as the closest approach distance. We could not obtain a closest approach distance for one trial in which the magpies flew out of the observation area and out of sight, which was therefore excluded from the analyses.

We conducted 101 trials (56 ground predator trials, 45 aerial predator trials) of which five ground and two aerial predator trials had to be excluded because a cat or bird of prey was present during the trial (see Figure 3.A-1 for the distribution of trials across the study area and Table 3.A-1 for an overview of number of trials per predator type, social environment and trial period).

(3.3.3) Social environment

We determined the social environment, i.e. single magpies, magpie pairs & families, and flocks of magpies, based on the number and behaviours of magpies present during the trial. If only one magpie was seen during the entire trial, it was scored as 'single' (9 and 16 trials in the non-breeding and breeding season, respectively). In three cases, another magpie appeared after the focal magpie had flown out into a different direction, so they were scored as two single magpies. Magpie ‘pairs’ (16 and 10 trials in the non-breeding and breeding season, respectively) could be identified because they stayed closer together and arrived in or left the trial area within one minute after each other, coming from and moving in similar directions. In eight cases, two or three pairs could be observed in distinct areas within the trial area, and conflicts with chasing arose if one pair came too close to the other. They were therefore scored as pairs. In addition, two trials were scored as pair even though an additional magpie was present in the study area, at greater distance from the pair. During the breeding season, ‘families’ (17 trials) were defined as two adult magpies staying closer together, with nearby fledglings identified based on begging behaviour**,** very short tail, dull head, and/or clean and shiny plumage (Birkhead 1991). For the pairs in the breeding season, we did not know if they still had nestlings, their offspring just dispersed or if they did not have a successful nesting attempt. For this reason and because there were no families in the trials conducted in the non-breeding season, we grouped pairs and families into one category for most analyses (‘pairs & families’). When more than one magpie was observed in a trial area at the same time and they did not classify as pair or family, they were scored as ‘flock’ (26 trials). Those were loose assemblies of at least two magpies that moved about mostly independently, at times closer to each other or further away, without avert aggression between individuals, and arriving in and leaving the trial area mostly independently. Three trials were excluded because we were not certain if the social environment classified as family or flock.

(3.3.4) Acoustic analyses

Trial recordings were loaded into Audacity® v3.2.4 (Audacity Team, 2023) to label chatters based on visual inspection of spectrograms and listening (see Chapter 2.3 for details). Chatters that were faint or that were commented as “distant” (i.e. out of the trial area) by the observers were labelled as such and excluded from the analyses. All labels with corresponding start and end times were exported as text files and loaded and merged with trial information in R v4.3.2 (R Core Team 2022) to extract the total number of chatters per trial.

(3.3.5) Statistical analyses

To understand the underlying reasons for which magpies alarm call, we determined how the anti-predator responses differed between different social environments and different predator types (Table 3.1). We therefore created generalised linear models using the package glmmTMB v1.1.4 (Brooks *et al.* 2017) in R v4.2.2 (R Core Team 2022) to test for an effect of the social environment (single, pairs & families and flock) and predator type on a) whether or not focal magpies emitted alarm calls (proportion of trials with chatters), b) the number of chatters per trial, and c) the closest approach distance to the model (details below). We then compared the estimated marginal (least-square) means between the social environments, i.e. 'single' versus 'pairs & families' versus 'flocks', with the package emmeans v1.8.2 (Lenth 2022). In addition, we created models on subsets of the data to further explore if season (non-breeding season and the breeding season) or fledgling presence in the breeding season affected the three above-mentioned behaviours of pairs.

For all models, we used the DHARMa package v0.4.6 (Hartig 2022) to assess model fits and the performance package v0.10.5 (Lüdecke *et al.* 2021) to ensure the explanatory variables were not collinear (all VIF < 2.5; (Johnston *et al.* 2018). We selected generalised linear models with a binomial family distribution and a logit link function for the likelihood that magpies chattered. For analyses of the number of chatters, we selected the negative binomial 1 family distribution with a log link function. We selected a Gaussian family distribution for the square-root transformed closest approach distance of magpies to the model. Social environment (or, in the subset models, either season or whether or not fledglings were present), predator type, the square-root transformed start distance and the trial period were set as fixed factors in all models, and the number of magpies present in a trial was set as additional fixed factor in models on chatter probability and number of chatters. We further included the interaction between predator type with social environment (season or fledgling presence for subset models) if a model comparison with anova chi-square tests showed that it significantly improved the model. If this was the case, we created separate models for the two threat types. We tested for the overall effect of each fixed factor using Anova type II (Wald Chisquare tests) in the glmmTMB package v1.1.8 (Brooks *et al.* 2017).

(3.3.6) Ethical note

We followed the *Guidelines for the treatment of animals in behavioural research and teaching* (ASAB/ABS 2020) to plan the experiments with a minimum impact on the tested birds. Eurasian magpies are not endangered in the Netherlands and we did not catch them. We conducted trials (ca. 20 mins in total) in urban areas where magpies frequently encounter humans, cyclists, cars, and predators like domestic cats. We limited the trial duration and kept the models covered between trials to minimise the level of distress to the birds. We further kept a distance of minimally ten meters to adult and fifteen meters to fledgling birds not to stress them.

(3.4) Results

(3.4.1) Likelihood of chatters (a)

Single magpies rarely emitted chatter calls when facing a predator model (Figure 3.1.a). In contrast, magpie flocks and pairs & families emitted chatter calls in many trials (Figure 3.1.a), and in significantly more trials with pairs & families than with single magpies (Table 3.2.a). The interaction of social environment and threat type did not significantly improve the model ($\chi^2_2 = 0.80$, $P = 0.669$, ΔAIC = 3.2). Pairs (or families) encountering a predator in the breeding season were more likely to chatter than those in non-breeding season (Figure 3.2.a, Table 3.3.i.a), but the presence of fledglings did not influence the probability that magpies chattered (Figure 3.2c, Table 3.3.ii.a). The interaction with threat type did not improve the latter two models (model comparison for season: $\chi^2_1 = 1.61$, $P = 0.204$, ΔAIC = 2.4; for fledgling presence: $\chi^2_1 = 0.33$, $P = 0.566$, ΔAIC = 1.6).

(3.4.2) Chatter rates (b)

Magpies greatly varied the number of chatters emitted, and this was determined by their social environments and the threat type they encountered (Figure 3.1.b): pairs & families chattered substantially more than single individuals, and slightly but significantly more than flocks (Figure 3.1.b, Table 3.2.b). Despite the significantly higher chatter rate towards ground predators than aerial predators (Figure 3.1.b, Table 3.2.b), the interaction between social environment and threat type did not improve the model (model comparison: $\chi^2_2 = 1.37$, $P = 0.504$, ΔAIC = 2.6). Magpies in pairs & families chattered at significantly higher rates during the breeding season than during the non-breeding season (Figure 3.2.b, Table 3.3.i.b), but the presence of fledglings did not affect the number of chatters during a trial (Figure 3.2.d, Table 3.3.ii.b). The interactions with threat type did not improve both latter models (model comparison for (i) season: $\chi^2_1 = 2.27$, $P = 0.132$, ΔAIC = -0.2; for (ii) fledgling presence: $\chi^2_1 = 0.31$, $P = 0.580$, ΔAIC = 1.7).

(3.4.3) Closest approach distance (c)

Magpies in different social environments differed in how closely they approached the model, yet in interaction with predator type (Figure 3.1.c, Table 3.2.c, model comparison with and without interaction: $\chi^2_2 = 8.47$, $P = 0.015$, ΔAIC = -4.5). In the subset including trials with ground predator trials only, magpie pairs & families approached the model significantly more closely than flocks and single magpies (Table 3.2.c). Magpies seemed to avoid close encounters with aerial predators in most trials (Figure 3.1), but single magpies stayed significantly further away from such predators than flocks (Table 3.2.c).

The approach distance of magpie pairs & families did overall not differ between the breeding season and non-breeding season (Figure 3.2.c, Table 3.3.i.c), although they stayed further away from aerial predators and the latter seemed particularly the case in the non-breeding season (but the interaction between threat type and season did not improve the model; $X^2_1 = 2.23$, $P = 0.135$, ΔAIC = -0.2). In the breeding season, the presence of fledglings did not make magpies approach the predator more closely (Figure 3.2.e, Table 3.3.ii.c) and the interaction with threat type did not improve the model ($X^2_1 = 0.09$, $P = 0.771$, ΔAIC = 2.0).

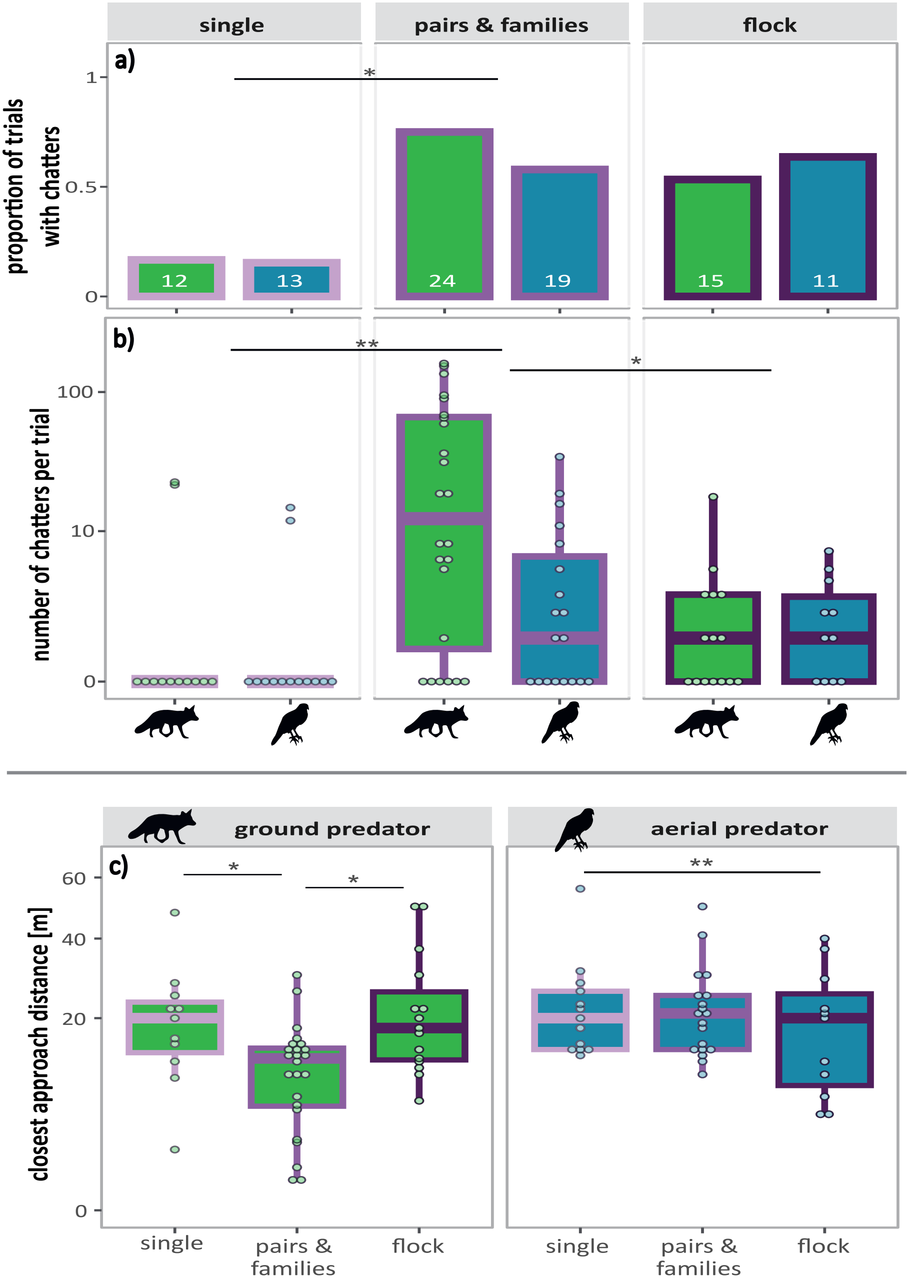


Figure 3.1. Magpie responses to different predator types (x-axes), separately for different social environments (columns). Displayed are (a) the proportion of trials in which chatters occurred, with the number of trials given in bars; (b) the number of chatters per trial (points) and boxplots per social environment and threat type displaying interquartile range (box), median (bold line) and the range of the data within 1.5 times the interquartile range (whiskers) on a log scale. (c) Closest approach of magpies to the taxidermic models of different predator types (columns), separately for different social environments (columns), on a square-root scale. Significant pairwise differences between social environments are indicated through horizontal lines (* *P* < 0.05, ** *P* < 0.01).

Table 3.2. Model results for magpie responses to predator models, giving the model estimates with standard errors and Anova (type II Wald chi-square tests) statistics of all fixed factors in the final models, as well as post-hoc pairwise comparisons of social environments. Sample sizes of the final models are given below the statistics. Because the interaction of predator type and social environment was significant for models of closest approach distance, we report values for (c.i) ground predator trials and (cii) aerial predator trials separately. Significant P-values (P < 0.05) are indicated in bold.

	a) Likelihood to chatter in a trial			**b) Number of chatters per trial**			**c.i) Closest approach distance to the ground predator model per trial**			**c.ii) Closest approach distance to the aerial predator model per trial**		
	estimate (SE)	**test values**	***P***	**estimate (SE)**	**test values**	***P***	**estimate (SE)**	**test values**	***P***	**estimate (SE)**	**test values**	***P***
intercept	-1.91 (0.87)			0.92 (0.62)			4.39 (0.46)			5.06 (0.22)		
social environment		$\chi^2_2 = 6.52$	**0.038**		$\chi^2_2 = 12.68$	**0.002**		$\chi^2_2 = 10.37$	**0.006**		$\chi^2_2 = 7.46$	**0.024**
… single - pair & families*	-1.94 (0.77)	$z = -2.53$	**0.013**	-1.59 (0.55)	$z = -2.87$	**0.004**	1.18 (0.46)	$t_{44} = 2.56$	**0.014**	0.22 (0.23)	$t_{37} = 0.97$	0.340
… single - flock*	-1.70 (1.04)	$z = -1.63$	0.106	-0.79 (0.70)	$z = -1.12$	0.263	-0.05 (0.56)	$t_{44} = -0.09$	0.926	0.75 (0.28)	$t_{37} = 2.72$	**0.010**
… pairs & families - flock*	0.25 (0.69)	$z = 0.36$	0.722	0.80 (0.41)	$z = 1.96$	**0.0496**	-1.23 (0.47)	$t_{44} = -2.59$	**0.013**	0.53 (0.27)	$t_{37} = 1.97$	0.057
predator type (aerial - ground)	-0.60 (0.52)	$\chi^2_1 = 1.33$	0.248	-0.93 (0.3)	$\chi^2_1 = 9.54$	**0.002**	-	-	-	-	-	-
trial period (2022 - 2021)	1.77 (0.65)	$\chi^2_1 = 7.38$	**0.007**	1.03 (0.32)	$\chi^2_1 = 10.36$	**0.001**	-0.14 (0.41)	$\chi^2_1 = 2.05$	0.152	-0.54 (0.22)	$\chi^2_1 = 5.77$	**0.016**
number of magpies	0.88 (0.38)	$\chi^2_1 = 5.41$	**0.020**	0.54 (0.16)	$\chi^2_1 = 11.32$	**0.001**	-	-	-	-	-	-
start distance	0.16 (0.25)	$\chi^2_1 = 0.41$	0.522	-0.07 (0.13)	$\chi^2_1 = 0.29$	0.593	0.26 (0.18)	$\chi^2_1 = 10.37$	**0.006**	0.83 (0.10)	$\chi^2_1 = 64.92$	**< 0.001**
number of trials	25 single, 43 pairs & families, 26 flocks			25 single, 43 pairs & families, 26 flocks			11 single, 24 pairs & families, 15 flocks			13 single, 19 pairs & families, 11 flocks		

* post-hoc pairwise comparison

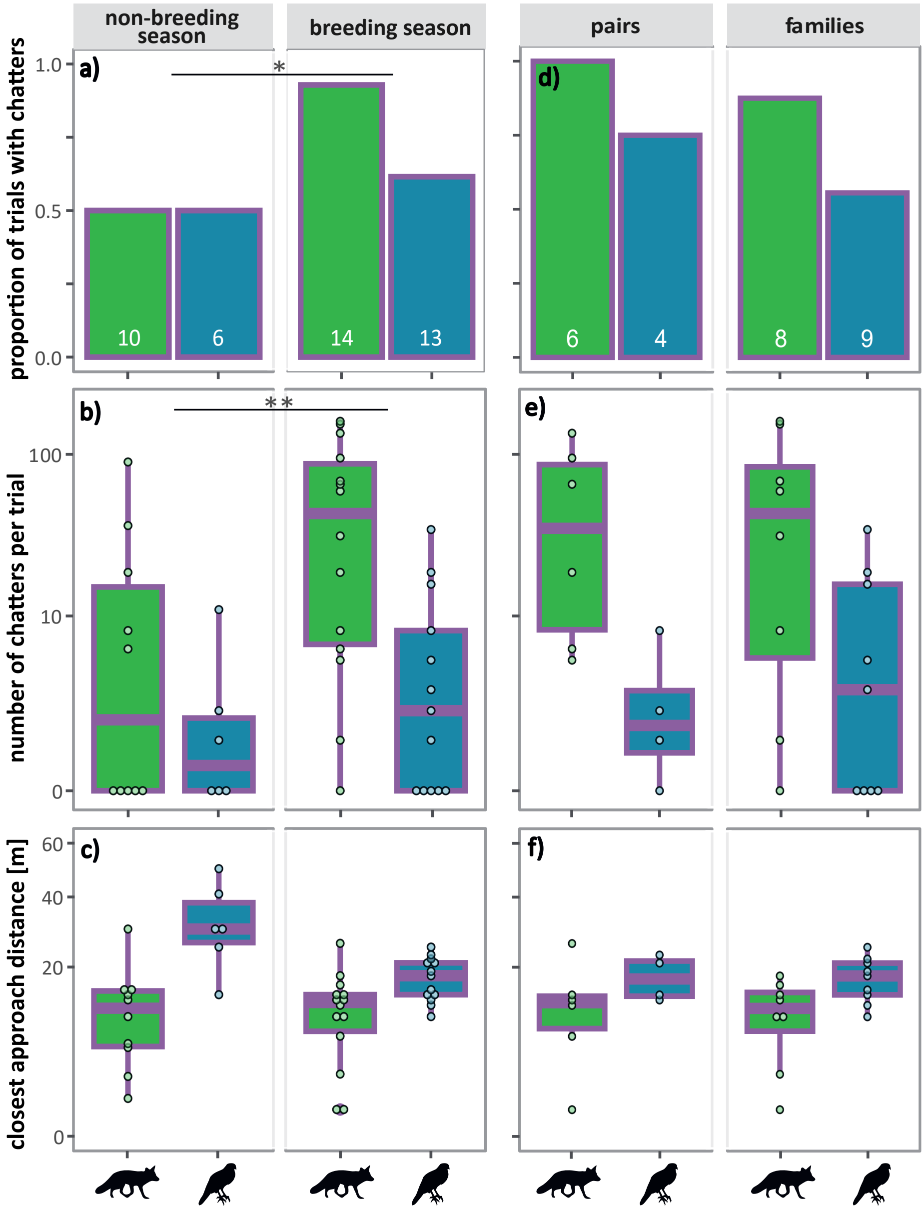


Figure 3.2. Magpie responses to different predator types (ground predators in green; aerial ambush predators in blue), separately for different seasons (a - c:) or social environments (d - f). Displayed are: (a & d) the proportion of trials in which chatters occurred; (b & e) the number of chatters per trial (log scale); and (c & f) the closest approach of magpies to the taxidermic model (square root scale). Dots reflect one trial and boxplots display interquartile range (box), median (bold line) and the range of the data within 1.5 times the interquartile range (whiskers) on a log scale. Numbers in (a & d) reflect the number of trials in each category. Significant pairwise differences between social environments are indicated through horizontal lines (* $P < 0.05$, ** $P < 0.01$).

Table 3.3. Results for ii. how magpie pairs and families differ between seasons or iii. how magpie pairs differ from families in anti-predator responses (a, b and c), giving the model estimates and Anova (type II Wald chi-square tests) of the selected models. Significant p-values ($P < 0.05$) are indicated in bold.

i. Magpie pairs & families between seasons									
	a) likelihood that magpies alarm called			**b) number of chatters per trial**			**c) closest approach distance to the model per trial**		
	estimate (SE)	**test values**	***P***	**estimate (SE)**	**test values**	***P***	**estimate (SE)**	**test values**	***P***
(intercept)	0.41 (0.60)			2.67 (0.42)			3.45 (0.27)		
season (breeding - non-breeding)	1.53 (0.77)	$\chi^2_1 = 3.93$	**0.047**	-1.09 (0.38)	$\chi^2_1 = 8.34$	**0.004**	-0.42 (0.32)	$\chi^2_1 = 1.71$	0.191
predator type (aerial - ground)	-1.10 (0.79)	$\chi^2_1 = 1.96$	0.161	1.07 (0.41)	$\chi^2_1 = 6.93$	**0.008**	1.29 (0.32)	$\chi^2_1 = 16.54$	**< 0.001**
number of magpies	0.47 (0.40)	$\chi^2_1 = 1.37$	0.242	0.39 (0.16)	$\chi^2_1 = 5.88$	**0.015**	-	-	-
start distance	0.34 (0.38)	$\chi^2_1 = 0.80$	0.371	-0.02 (0.17)	$\chi^2_1 = 0.02$	0.899	0.49 (0.16)	$\chi^2_1 = 9.56$	**0.002**
number of trials	16 in autumn, 27 in summer			16 in autumn, 27 in summer			16 in autumn, 27 in summer		
ii. Magpie pairs versus families in summer 2022									
(intercept)	4.89 (2.04)			3.88 (0.36)			3.26 (0.33)		
social environment (pairs - families)	-2.71 (1.64)	$\chi^2_1 = 2.71$	0.099	-0.19 (0.39)	$\chi^2_1 = 0.24$	0.626	-0.23 (0.38)	$\chi^2_1 = 0.35$	0.556
predator type	-2.08 (1.35)	$\chi^2_1 = 2.37$	0.124	-1.35 (0.43)	$\chi^2_1 = 9.76$	**0.002**	1.01 (0.36)	$\chi^2_1 = 7.79$	**0.005**
number of magpies	1.69 (1.10)	$\chi^2_1 = 2.36$	0.124	0.45 (0.17)	$\chi^2_1 = 6.86$	**0.009**	-	-	-
start distance	-0.14 (0.63)	$\chi^2_1 = 0.05$	0.829	-0.26 (0.21)	$\chi^2_1 = 1.53$	0.215	0.26 (0.19)	$\chi^2_1 = 1.98$	0.159
number of trials	10 pairs, 17 families			10 pairs, 17 families			10 pairs, 17 families		

(3.5) Discussion

Our results, based on simulated predator encounters, show that magpies varied the intensity of alarm calling according to which individuals were around. In absence of an audience of conspecifics, magpies rarely alarm called, whereas in social settings they responded strongest when they were with their partner or in families with fledglings, especially in the breeding season. This variation in alarm calling across social environments, along with our assessment of approach behaviour and what types of predators induce alarm calls, enables us to make inferences about the function and the adaptive benefits of alarm calling in magpies, as we explain below per tested hypothesis.

(3.5.1) Pursuit deterrence for own survival

Our results strongly suggest that magpies do not alarm call to deter the predator from attacking (Caro 1995; Huang & Caro 2023). If 'pursuit-deterrence' is the main function, it can be expected that magpies would alarm call on encountering an ambush predator even when alone, to alert such predator that it has been detected (Caro, 1995; Shelley & Blumstein, 2005; Ostreiher 2023; Wheeler, 2008). However, single individuals rarely alarm called and did not approach predators, even though the ground predators and especially the aerial predators used in this study are ambush predators that may give up their hunt or move away when signalled at (Cresswell 1993; Holley 1993). Yet, magpies do not appear to use chatters to signal their perception at predators.

Results of studies testing the pursuit-deterrence hypothesis across species are mixed. Our result (and that of several other studies; e.g. Sullivan 1985; Krams et al. 2006, Wich & Sterck 2003) is different from other studies that show that alarm calling might function as pursuit-deterrent signal. For example, California ground squirrels (*Otospermophilus beecheyi*) use visual tail-flagging to signal their vigilance or perception at snake, which reduces the likelihood of snake strikes, and snakes move further away after mobbing displays (Clark 2005; Barbour & Clark 2012). Leopards (*Panthera pardus*) have also been shown to move away from alarm-calling primate groups (Zuberbühler *et al.* 1999; Isbell & Bidner 2016). In the case of ground predators, one may expect that birds likely do not need to signal to avoid attacks as they can just fly away or stay up out of reach of the predator, but single magpies also did not respond to sparrowhawks, which are notorious and agile ambush predator (Cresswell 1994a). One reason could be that sitting, rather than flying, sparrowhawks do not pose an immediate threat (Petracca & Caine 2013), but the result that magpies did alarm call in response to sparrowhawk models when an audience was present suggests that this is not the explanation for lack of effect for magpies without audience.

Our finding that magpies did not alarm call or approach predators when they were alone should not be interpreted as evidence that the alarm call of magpies does not function in chasing predators away. Magpies in other social settings mob predators for benefits such as saving others (see below) and the high call rates in such settings likely function to some extent to deter the predator (Carlson & Griesser 2022; Chapter 2). In this sense, when magpies are alone, they might not be motivated or not be able to chase predators away, and the former is likely the case. Single magpies are often floaters without a territory (given that pairs usually stay together, and a partner is replaced quickly after disappearing (Baeyens 1981a; Birkhead 1991), who may therefore lack the motivation to risk being attacked (by predators but also territory owners) while chasing predators away. Regardless, however, while magpie alarm calls may function in predator repellence, it can be concluded that alarm calls are not selected in magpies because of pursuit-deterrence increasing their survival chances.

(3.5.2) Immediate mutualism by alerting conspecifics

We found that magpies in flocks sometimes alarm called. However, this did not immediately lead to obvious responses by conspecifics that the caller may benefit from. This suggests that alarm calling in magpies does not, or at least not primarily, function to elicit a joint escape (Charnov & Krebs 1975; Cresswell 1994b), or recruit additional mobbers (Curio 1978; Caro 2005; Carlson & Griesser 2022). It has generally been hypothesised that if other individuals are around, calling out while fleeing may aid the caller if it elicits others to flee and potentially call out as well (Charnov & Krebs 1975). Such a response might confuse the predator, distract it from the first individual to move and call out, and dilute the risk for the individual (Hamilton 1971; Cresswell 1994b; Lehtonen & Jaatinen 2016; Hogan *et al.* 2017). Another reason for calling is that calls may recruit conspecifics to join in mobbing the predators, thereby diluting the risk to the individual and increasing the likelihood to deter the predator (Flasskamp 1994; Gursky 2006; Wheeler 2008). We expected to find such an effect, especially in trials where we presented sparrowhawks, as alarm calling in groups has been shown to decrease hunting success of sparrowhawks and to reduce the risk for an individual to be caught (Cresswell 1994a, b; Hogan *et al.* 2017). Accordingly, we observed that individuals in flocks sometimes alarm called when they encountered a predator. However, they did so to much lesser extent than individuals in pairs and families and regardless of the type of predator. More importantly, we observed neither intense mobbing behaviour nor joint escape or cacophonies of calls that might distract and confuse the predator in trials with flocks. Thus, the weak response of flocks to predators, combined with the absence of collective actions, suggests that immediate mutualistic benefits by alerting conspecifics poorly explain the evolutionary maintenance of alarm calling in magpies.

(3.5.3) Saving others

Considering that (1) pursuit-deterrence and (2) mutualistic benefits do not seem to explain alarm calling in magpies (see above), it is likely that alarm calling and mobbing function to warn or save particular conspecifics with whom individuals have a specific relationship. If so, alarm calling might be selected because it yields adaptive benefits that are based on (3.1) mutualistic benefits in groups (survival benefits), (3.2) family ties (kin-selected benefits), or (3.3) reproduction (direct fitness benefits).

(3.5.3.1) Delayed mutualism: protecting conspecifics for group-related benefits

In our study, individuals in flocks did not respond to predators at high rates, suggesting that benefits of group membership are not the main driver of alarm calling and mobbing in Eurasian magpies. While there is evidence that maintaining group benefits can explain alarm calling in species with stable groups (e.g. in cooperative breeders protecting group members; (Teunissen *et al.* 2020), see also (Cheney & Seyfarth 1985; Randall *et al.* 2000), magpie flocks are ephemeral and may not entail longer-term mutualistic benefits, such as communal roosting, enhanced finding food, and protection from predators through immediate mutualistic benefits. The benefits of flock membership are not well studied in magpies (Baeyens 1981a), but our results suggest that they are not strong enough to select for risk-taking behaviour of individuals when exposed to a predator. To what extent specific benefits of group living can explain variation in alarm calling and mobbing across species remains to be tested.

(3.5.3.2) Saving kin

In species where individuals associate with kin for prolonged periods, alarm calls might function to warn and protect non-descendent kin for indirect fitness benefits (‘kin selection’; Hamilton 1964; Maynard Smith 1965; Stephan & Zuberbühler 2021). Some evidence indeed exists for kin-directed alarm calling in other species (Dunford 1977; Hoogland 1983; da Silva *et al.* 2002; Wheeler 2008), though it is not always clearly differentiated from direct fitness benefits through protecting offspring (Sherman 1977; Chapman *et al.* 1990). Unfortunately, we do not have data on whether flocks consist of related individuals (note that we consider parents protecting offspring as ‘reproductive’ benefit; see below), but this might be the case since magpies often stay close to their natal territories, and siblings may join the same flock (Baeyens 1981a; Birkhead 1991). If so, this may explain the occurrence of alarm calls by individuals in flocks in some of our experimental trials, but this remains to be tested in flocks where relatedness between individuals is known. In any case, since the anti-predator responses in flocks are relatively weak, it seems unlikely that kin-selected benefits are the main driver in this species.

(3.5.3.3) Saving others for reproductive benefits: mate and offspring protection

In our experiment, by far the strongest response to predators was given by individuals in pairs and families, and call rates were higher during the breeding season than the non-breeding season. As such, alarm calling can be considered a form of parental care, where parents warn or save vulnerable offspring against predators (Zuberbühler 2009). Indeed, many other studies also found that mobbing is apparently driven by reproductive benefits, with some bird species mobbing predators mainly during the breeding season (Curio 1978; Shedd 1982; Regelmann & Curio 1983; Cully & Ligon 1986; Freeman & Miller 2018). Thus, alarm calls of magpies might function in saving offspring or, for example, in teaching offspring about danger (Graw & Manser 2007; Griesser & Suzuki 2017).

Interestingly, and unlike studies on some rodents that found higher alarm call rates after pup emergence (Blumstein *et al.* 1997; Randall *et al.* 2000), we found that alarm calling behaviour of pairs without fledglings in the breeding season was equally high as when fledglings were present. While this might reflect a ceiling effect in the response of individuals with fledglings (indeed alarm call rates were sometimes extremely high), several other non-exclusive explanations may apply for why individuals responded so strongly in the presence of only their partner. First, individuals in pairs and family groups are, unlike single magpies and flocks, year-round territorial and if alarm calling functions as predator repellence, only territorial individuals may be motivated to chase predators away for future safety in their territory (Trivers 1971; Buitron 1983; Pettifor 1990; Flasskamp 1994; Barbour & Clark 2012). However, it remains to be tested whether occasional repellence would lead predators to avoid the area in the long term. Second, aggression rates towards predators during the breeding season could generally be higher, for example because it is regulated by hormone levels, regardless of whether fledglings are (still) present (Cully & Ligon 1986). However, this does not explain why the response to predators by pairs was in some cases still relatively high during the non-breeding season (although the response of pairs to predators was weaker in the non-breeding season on average, some individuals emitted high alarm call rates and approached remarkably close to predators). Third, our results that individuals in pairs without fledglings alarm called at high rates seems to indicate that magpies do so to protect their partner (Witkin & Ficken 1979; Hogstad 1995; Zaccaroni *et al.* 2013; Stephan & Zuberbühler 2016). This may reflect some form of securing a partners' parental care in the breeding season (even though we did not observe fledglings, we could not fully rule out that nestlings were present). Alternatively, the benefit of saving a partner could also manifest on the longer term. For example, single magpies might be at risk of usurpation and eviction by an already formed breeding pair (Birkhead 1991) and, although replacing a partner while having a territory may not be difficult (Baeyens 1981a), retaining the current partner in this species with long-term pair bond may lead to efficient behavioural coordination and higher overall reproductive success, as it does in other species (Black 2001; Sánchez-Macouzet *et al.* 2014; Bebbington & Groothuis 2023). Consequently, magpies could derive benefits from protecting their partner, and long-term benefits of doing so might explain why magpies in pairs sometimes also showed strong response to predators outside the breeding season. Future investigations would be worthwhile to assess whether certain characteristics, such as year-round territoriality selecting for long-term pair bonds, can be important determinants for the expression of alarm calling in birds.

(3.5.4) Sexual selection: showing off or future reproduction

Recently, it has been suggested that animals may signal their quality, motivation or strength to (potential) partners by engaging in risky behaviour such as predator mobbing (Gursky 2006; Wilson *et al.* 2008; da Cunha *et al.* 2017b; Bebbington & Groothuis 2023). We found that individuals were more likely to alarm call and approach the predator when they were in a pair bond (see above). However, it might be difficult to disentangle display behaviour from direct partner protection in breeding pairs (Sullivan 1985; Krams *et al.* 2006). Indeed, fowl (*Gallus gallus*) males that alarm called more also had a subsequent higher mating success (Wilson *et al.* 2008) but females did not express a direct preference for alarm calling males in a subsequent study, suggesting that the finding was perhaps not causal (Wilson & Evans 2010). To circumvent this problem, we tested the prediction that risky anti-predator behaviour in magpie flocks would serve as common display behaviour because magpies often form pairs within flocks (Birkhead 1991). Nonetheless, we found that magpies in flocks did not display overt risk taking (e.g. attacking sparrowhawks), which leads us to conclude that alarm calling behaviour does not seem primarily shaped by sexual selection.

(3.5.5) Conclusion

By testing predictions of the various proposed functions and adaptive benefits of alarm calling, we conclude that alarm calling in magpies mainly functions to warn and/or protect a breeding partner (also outside the breeding seasons) and offspring for reproductive benefits. Alarm calling in magpies seems not, or to lesser extent, function to advertise perception or quality to predators, to gain mutualistic benefits from group responses to predators, to save beneficial group members or non-descendent kin, or to display individual quality to potential partners. In general, the wide variety in the function of alarm calling across species illustrates that the behaviour depends on different ecological factors, biology, and social environment of species. Identifying how these factors shape the relative importance of the various adaptive benefits of alarm calling across species would allow for a more generic conclusion of how the behaviour evolves despite its inherent costs.

(3.6) Acknowledgements

We thank Huiyi Li, Tim van den Bosch, and Arnout Lindeman for assisting in data collection and acoustic analyses.

(3.7) Appendix

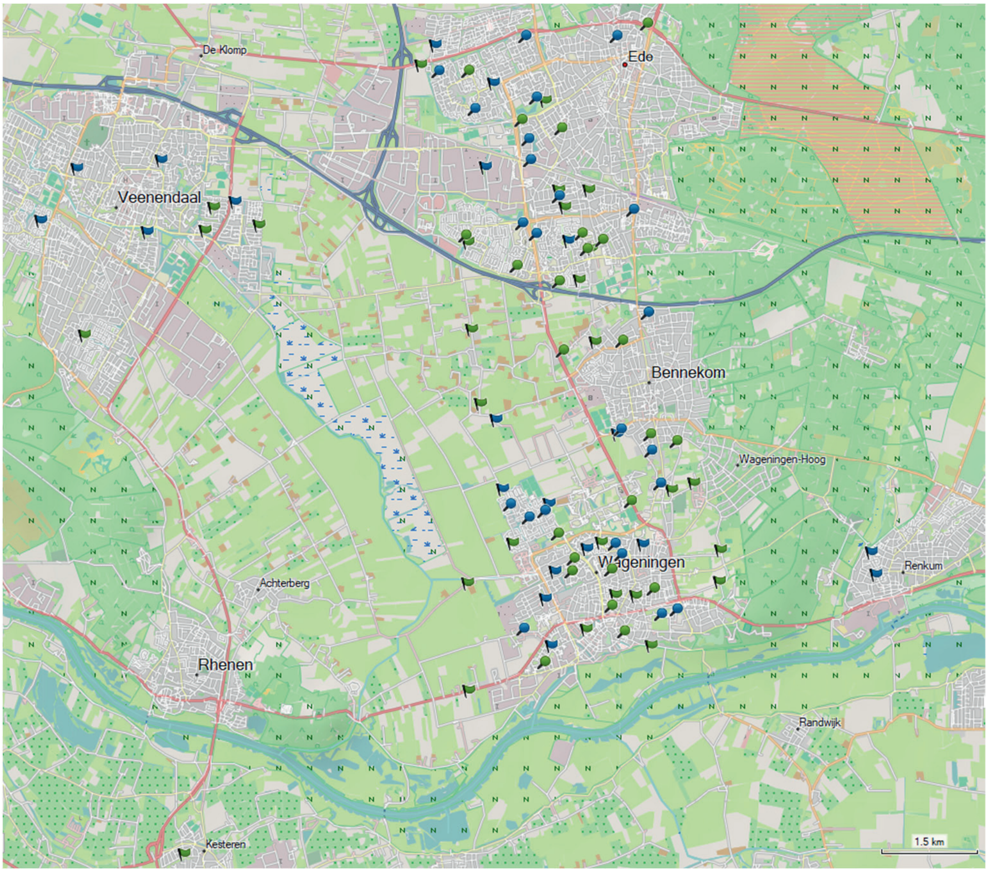


Figure 3.A-1. Overview map of the study area and trial locations (blue: aerial predator, green: ground predator trials) from the two trial periods (flags: autumn 2021, pins: summer 2022), created in Garmin® BaseCamp v4.7.4 (© 2008 – 2024 Garmin Ltd. or its subsidiaries), map data © OpenStreetMap contributors (ODbL), produced map © FZK project).

Table 3.A-1. Sample sizes given as number of trials per predator type, social environment and trial period.

	2021				**2022**			
	single	**flock**	**pair**	**family**	**single**	**flock**	**pair**	**family**
ground predator	4	14	10	0	8	1	6	8
aerial predator	5	9	6	0	8	2	4	9

Table 3.A-2. Model results testing for differences between predator model types and social environments in the start distance of the trial.

	χ^2	**df**	***P***
predator type$_a$	0.78	1	0.377
social environment$_a$	1.00	2	0.607
predator x social environment$_b$	1.78	2	0.411

a Anova type II Wald's Chi-square test on the model excluding the interaction

b anova chi-square test comparing the models with and without interaction

Ch. 4 | Pair-coordinated defence: Overlapping calls alter territorial responses in Eurasian magpies

Miriam Kuspiel, Sjouke A. Kingma, Heleen Vermeulen, Marc Naguib

(4.1) Abstract

Vocalisations are widely used to signal internal strength or motivation of a caller in competitive interactions. Most research on birds explored the function and variations of male song in territorial defence, so that much less is known about calls emitted by both males and females. However, males and females commonly call frequently in various contexts, including territorial conflicts and predator-induced situations. Such calling may be coordinated, thus leading to overlapping or alternating sequences of calling. This raises the question as to whether or not variation in timing of pair calling is perceived as meaningful by receivers. Here, we tested with playback experiments whether the Eurasian magpie (*Pica pica*), a species producing alarm calls (so-called chatter calls) in territorial defence, responds stronger to simulated pair-intruders who overlap their calls than to those who alternate them. Magpies emitted a significantly longer first chatter in response to overlapping playback, yet tended to approach the playback of alternating chatters (with consequently longer chatter bouts) more closely. These findings exemplify that the timing of pair calling matters, but in more complex ways than we predicted. The overlapping playback appears to trigger a stronger initial chatter response but a weaker approach response, suggesting that the response measures reflect different levels of arousal or response strategies. These findings expand on classical experiments on call function, showing that pairs can vary the message by coordinating their alarm calls in different ways, similar to how duetting pairs time their song contributions in advertisement signalling.

KEYWORDS: Territorial intrusion, alarm call, cooperation, female calls, corvid.

(4.2) Introduction

Many animals use vocal signals as territory advertisement. By providing information about a caller's quality or motivation to defend a resource, conflicts may be avoided or settled through acoustic signalling without costly physical interactions (Maynard Smith & Price 1973). In birds, male song is the most apparent and most studied territorial signal, where song rate, song matching and overlapping an opponent's song have been associated with caller characteristics (e.g., condition), the motivation to escalate a conflict (i.e., aggressiveness), or the strength of a signal (inferred from how strongly resident birds respond to the intruder's signal) (Todt & Naguib 2000; Vehrencamp 2000; Gil & Gahr 2002; Collins 2004; Catchpole & Slater 2008; Baker *et al.* 2012; Logue 2021). Yet, song is not the only acoustic signal produced by birds.

The possibly most common vocalisations are calls, including alarm calls given by both sexes. These are also frequently produced in aggressive contexts with conspecifics (Morton 1977; Baker *et al.* 2012; Digweed 2019; Strauß *et al.* 2020). In great tits (*Parus major*), higher levels of aggressiveness and approaching a simulated intruder have been shown to be associated with calls more so than song (Araya-Ajoy & Dingemanse 2014). In some corvids, the song unlikely functions as far-range territorial signal due to its low amplitude, so that other vocalisations are probably used for territorial defence (Brown 1985; Martin *et al.* 2024). This highlights the wider function of male and female alarm calls in communication including territorial defence.

Alarm calls are primarily studied in predatory contexts, testing for context-specificity of call types and threat urgency-coding in call structures and rates (Blumstein 1999b; Townsend & Manser 2013; Suzuki 2016). For instance, higher alarm call rates may encode a greater urgency of a threat and a more aroused caller (Blumstein 1999b), which could signal a more aroused, motivated or aggressive territorial intruder (Morton 1982). Moreover, alarm calls in many species are given not only by one individual in a group but by several individuals (e.g. Arabian babblers *Turdoides squamiceps* (Naguib *et al.* 1999b; Sommer *et al.* 2012). The timing and usage of calls by multiple individuals inevitably affects the signalling pattern of the entire pair or group as perceived by the receiver. Hence, a receiver may respond differently depending on whether these calls are overlapping another or are alternating even if all individuals call independent of each other. Nonetheless, the perceived threat of temporal coordination of multiple alarm callers has been tested in neither predatory nor conspecific interaction contexts as far as we know.

Vocal coordination has been studied primarily in duetting bird species, where male and female pair members coordinate their vocalisations in duets, usually to defend their territory (Todt & Naguib 2000; Hall 2004, 2009; Dahlin & Benedict 2014; Logue & Hall 2014). While most duetting species alternate their vocalisations in rapid alternations, Carolina wren (*Thrythorus ludovicianus)* females, for example, duet by overlapping the male song with occasional trills, thereby creating an overlapping joint signal (Price *et al.* 2023; Zapata & McEntee 2023). Overlapping vocalisations may lead to a high signal to noise ratio and thus be audible over a longer distance (de Araújo *et al.* 2011; Rehberg-Besler *et al.* 2016). Furthermore, playback experiments have shown that coordinated duets rather than solo song, and even the timing accuracy of coordinated duets, can be perceived as a greater threat and elicit a stronger response by the resident birds (Hall 2004; Hall & Magrath 2007). We explored if overlapping alarm calls in territorial contexts may similarly alter the strength of the total signal and thus the response of the resident birds as found for temporal coordination in duetting species.

Eurasian magpies are an ideal model to test these ideas, as pairs defend their territory together and both sexes emit high rates of harsh and loud “chatter” alarm calls during simulated territorial intrusions (Chapter 2; Baeyens 1981b). The three to 124-syllable calls of pairs often overlap (Chapter 2) so that conspecifics may perceive the total chatter response differently when intruding pairs alternate or overlap their calls. We therefore tested if playbacks of overlapping chatter calls elicit a stronger territorial response than playbacks of alternating chatter calls in magpies, as predicted if overlapping calls are perceived as a stronger signal. We predicted that territory owners respond faster and stronger (i.e. with more or longer chatter calls, overlapping chatter calls, or approach more closely) to playbacks of overlapping chatter calls than alternating chatter calls.

(4.3) Methods

(4.3.1) Study area

Eurasian magpies occur at high densities in our study area in and around Wageningen (N51° 58.489' E5° 39.901'). Breeding pairs defend all-purpose territories year-round, where responses towards territorial intrusions are strongest during the late breeding season (May to July; Baeyens 1981b). Nest building already commences in autumn and nests from previous years are often re-used, so we mapped 265 magpie nests in Wageningen in the winter preceding our experiments when the large nests are conspicuous in leafless deciduous trees. We conducted playback experiments at 40 sites from 24 May to 13 June 2022, when magpie nestlings start to fledge (Birkhead 1991). Because magpies were unringed in our study area, we maintained minimally 200 m between experimental sites to avoid testing the same pair in two sites (based on neighbour-nest distances in European magpie studies with similarly high breeding densities; Birkhead 1991; Lehmann *et al.* 2005). We conducted experiments in the mornings (between 08:00 and 11:30 hours).

(4.3.2) Call recordings and playback file construction

Chatter calls were recorded with a Sennheiser ME66/K6 microphone connected to a Tascam DR-100 MK III audio recorder (recording settings: 44 kHz, 16 bit) during simulated territorial intrusions, in which we placed taxidermic models of magpies in sight of magpies encountered in our study area in summer 2020, autumn 2021, and summer 2022 (see Chapter 2 for details). We selected 88 chatter calls (sex of callers unknown) that were at least six syllables long and did not overlap with background noise (selected from recordings at 18 locations, one to 11 calls per location). Chatter calls were cut to six syllables if longer, high-pass filtered at 300 Hz with 48 dB roll-off, normalised to an amplitude of -1 dB, and fade-in and fade-out effects were applied up to the start of the first syllable and from end of the last syllable (over 0.02 to 0.03 s) in Audacity® v3.1.3 (Audacity Team, 2023). We created one-minute playback files by repeating a call from one location at natural and variable inter-call intervals (0.5-5 s) on the left channel and a call from another location on the right channel, either (a) overlapping the calls on the left channel for the last three or four syllables or (b) starting 0.03-0.14 s after the left channel calls (17 chatters per channel) so that one channel was leading the interaction (Naguib & Todt 1997; Naguib *et al.* 1999a). The inter-call intervals were kept silent. We exported playback files as wav files and played them back on two UE BOOM speakers (version 2) which were paired as stereo and connected via Bluetooth to the mobile phone of the experimenter. The peak sound pressure level of playbacks ranged from 84 to 97 dB (measured from a 1 kHz sine wave normalized to the same peak amplitude as the chatter calls with a Voltcraft SL300 SPL meter (A-weighted, fast) at one meter distance to the loudspeaker (each measured separately) on different days outside with full or low battery power of the loudspeakers). Each playback was used at only one location and different chatter combinations were used at the same location. We maintained minimally 200 m between the playback location and the locations where the corresponding chatters were recorded to ensure that we did not use a focal magpies' own call (based on neighbour-nest distances in magpie studies with similarly high breeding densities; Birkhead, 1991; Lehmann et al., 2005).

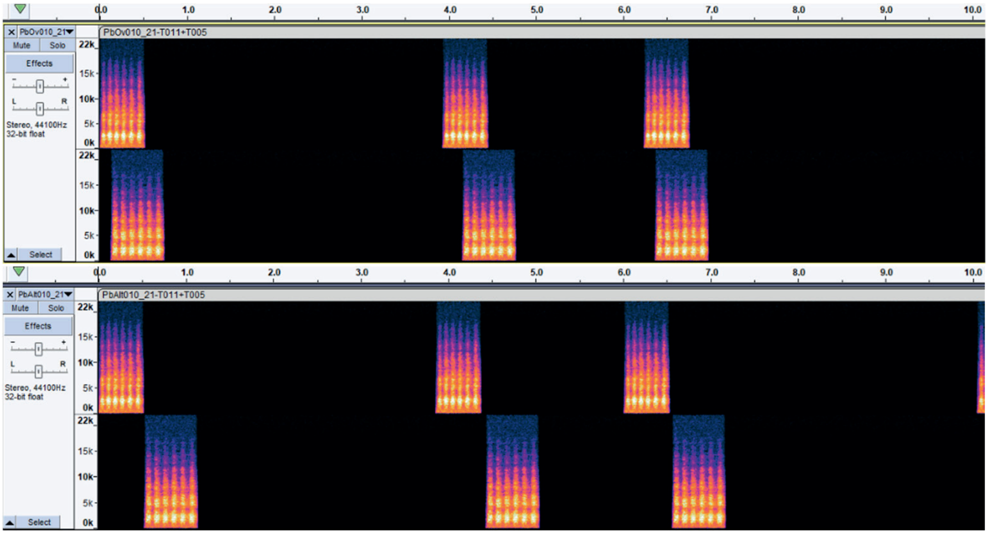


Figure 4.1. Sound spectrograms of the first ten seconds of exemplary alternating and overlapping chatter playbacks displayed in Audacity® v3.2.4 (Audacity Team, 2023), with the frequency in Hz on the y-axis and the time in seconds on the x-axis (above the spectrograms). Colours indicate the sound pressure level of a given frequency at a given time, ranging from black over purple to bright yellow (normalised to -1 dB).

(4.3.3) Experimental setup

We followed a paired design and broadcast both (a) overlapping and (b) alternating playbacks at each of the 40 playback sites, with two to five days between experiments at the same location (to prevent habituation effects in magpie responses). The order of treatments was set randomly with the restriction that the same playback type (overlapping or alternating) was not used in more than two consecutive experiments a day.

We placed the loudspeakers in a shrub or tree at a selected playback site. Due to limited available shrubs or access to the nest tree at many playback sites, the loudspeakers were placed at 19 ± 10 m (mean ± SD) from the nest tree and at 1.3 ± 0.3 m (mean ± SD) distance from each other. Loudspeakers were placed in the exact same location in the second trial at a playback site. To start a trial, we started the one-minute playback from 19 ± 4m (mean ± SD) distance to the loudspeakers (limited by visibility, urban traffic, and range of the Bluetooth signal). During the subsequent three minutes, we noted on audio recorders when magpies arrived in the trial area, how many magpies were present, and how closely they approached the loudspeaker (estimated with a mark at 5 m from one of the loudspeakers and measured post-trial with a tape measure or range finder (Leica rangemaster 2400) between the approach location closest to one of the loudspeakers). We recorded magpie vocalisations with a Sennheiser ME66/K6 microphone connected to a Tascam DR-100 MK

III recorder (recording settings: 44 kHz, 16 bit). When no magpies were seen or heard during the playback (i.e., during the first minute of the trial, when we could be sure that magpies perceived the stimulus), we waited five minutes and performed the same playback experiment again (for those, the responses to the second playback round were used for the analyses).

(4.3.4) Acoustic analyses

We used Audacity® v3.2.4 (Audacity Team, 2023) to label trial starts, magpie arrivals and departures, chatters (labels set over the entire single chatter or overlapping chatter sequence, containing syllable counts of single chatters or the number of chatters that overlap in a sequence) and disturbances (e.g. cats appearing in the trial area) based on listening to the recordings of vocalisation and notes and inspecting the spectrogram (Hann window with 1024 FFT length and zero padding factor 2, linear scale, 20 dB gain and 80 dB range). When playback chatters could not be clearly distinguished from chatters of focal magpies, we aligned the playback file with the recordings.

All labels (including label start and end times) of a trial were exported as text file and merged with trial information in R v4.3.2 (R Core Team, 2023). We used the packages tidyr v1.2.0 (Wickham & Girlich 2022) and dplyr v1.0.8 (Wickham *et al.* 2022) to extract for each trial a) the closest approach distance of magpies to the speakers, which we square-root transformed to give greater weight to differences in distances close-by (e.g., one versus two meters) than further away (e.g., 30 versus 31 meters), b) the total number all chatter calls (including those that overlap with another) and total duration of chattering (i.e. total duration of all single chatter calls and overlapping chatter sequences), and c) the number of overlapping chatter sequences. For those trials in which chattering occurred, we further extracted d) the latency between playback start and the first chatter and e) the number of syllables of the first chatter. In two trials (at the same trial location), a cat appeared during the trial inducing a strong anti-predator response, so all subsequent chatters were excluded from the analyses.

(4.3.5) Statistical analysis

We used generalised linear mixed models with the R package glmmTMB v1.1.8 (Brooks *et al.* 2017) to test for differences in magpie responses (a-e) to overlapping versus alternating chatter playbacks. Since we conducted both playback types sequentially at the same site, we included the playback site as random effect and trial sequence as fixed factor in all models. We used the Dharma package v0.4.6 (Hartig 2022) to assess model fits and violations of assumptions (e.g. overdispersion) and the performance package v0.10.5

(Lüdecke *et al.* 2021) to test for collinearity (all VIF < 2.5) between predictors (Johnston *et al.* 2018). We chose family distributions for each model (see below) based on data type (binomial, count or continuous) and for which model assessment plots best fit assumptions for linear models.

Because we could not be certain if any magpies actually heard the playback when no magpies appeared during the playback, we excluded those trials from all analyses (8 alternating playback trials and 12 overlapping playback trials; playback type did not significantly affect whether magpies appeared during playback, $P = 0.27$, Table 4.A-1.a).

(a) *closest approach distance*. To test the effect of playback type on the square-root transformed closest approach distance, we used a Gaussian family distribution and excluded two trials where the closest approach was not clear due to dense vegetation (remaining with 31 alternating and 27 overlapping playback trials at, in total, 35 distinct playback sites).

(b) *number of chatters*. To test the effect of playback type on the total number of all chatter calls, we used a negative binomial 1 family distribution with a log link function. Even though the duration of a chatter call can vary greatly (2-56 syllables), the number of chatters was highly correlated with the total duration of chattering (Kendall's tau = 0.92, $z = 9.20$, $P < 0.0001$). We therefore analysed only the effect of playback type on the number of chatters (see Table 4.A-2.b for analyses on the total duration of chattering). We excluded two trials in which a cat appeared during a trial (remaining with 31 alternating and 27 overlapping playback trials at, in total, 35 distinct playback sites). In addition to the co-variables listed above, we included the number of magpies present in a trial (to account for multiple potential callers) as fixed factor.

(c) *number of overlapping chatters*. To test the effect of playback type on the number of overlapping chatter sequences, we used a negative binomial 1 family distribution with a log link function and included the number of magpies present in a trial as fixed factor in addition to the co-variables listed above. We excluded two trials in which a cat appeared during the trial (remaining with 31 alternating and 27 overlapping playback trials at, in total, 35 distinct playback sites).

(d) *latency to chatte*r. To test the effect of playback type on the latency to the first chatter call we used a Gaussian family distribution. Trials without chatter calls were excluded from this model (remaining with 23 alternating and 15 overlapping playback trials at, in total, 27 playback sites).

(e) *number of syllables*. To test the effect of playback type on the number of syllables of the first chatter call, we used a negative binomial 2 family distribution with a log-link function and excluded trials without chatters (remaining with 23 alternating and 15 overlapping playback trials at, in total, 27 playback sites).

We extracted test statistics for the effects of playback type and fixed co-variables in all models with Anova Type II analysis-of-variance tables for model objects (rstatix package v0.7.0, Kassambara, 2021). Correlations between all response variables are given in the appendix (Table 4.A-2)

(4.4) Results

Magpies responded differently to playbacks of alternating versus overlapping chatters (Figure 4.2, Table 4.1). Magpies approached the loudspeaker more closely in trials with alternating than overlapping playback at 16 playback sites and vice versa at only 4 playback sites (Figure 4.2a), resulting in a marginally non-significant (*P* = 0.055) closer approach to the loudspeaker in alternating playback trials (Table 4.1.a). Magpies also more often emitted more chatters in trials with alternating playback (ten sites) compared to overlapping playback (five sites) (Figure 4.2.b), yet this difference was not significant (Table 4.1.b). Magpies also did not differ in the number of overlapping chatter sequences between trials with alternating and overlapping chatter playback (Figure 4.2.c, Table 4.1.c).

In those trials in which magpies chattered, the latency to emit the first chatter did not differ between playback types (Figure 4.2.d, Table 4.1.d). However, the duration of the first chatter towards alternating playbacks consisted of significantly fewer syllables (median: 9 syllables) than first chatters towards overlapping chatter playbacks (median: 15 syllables, Figure 4.2.e, Table 4.1.e).

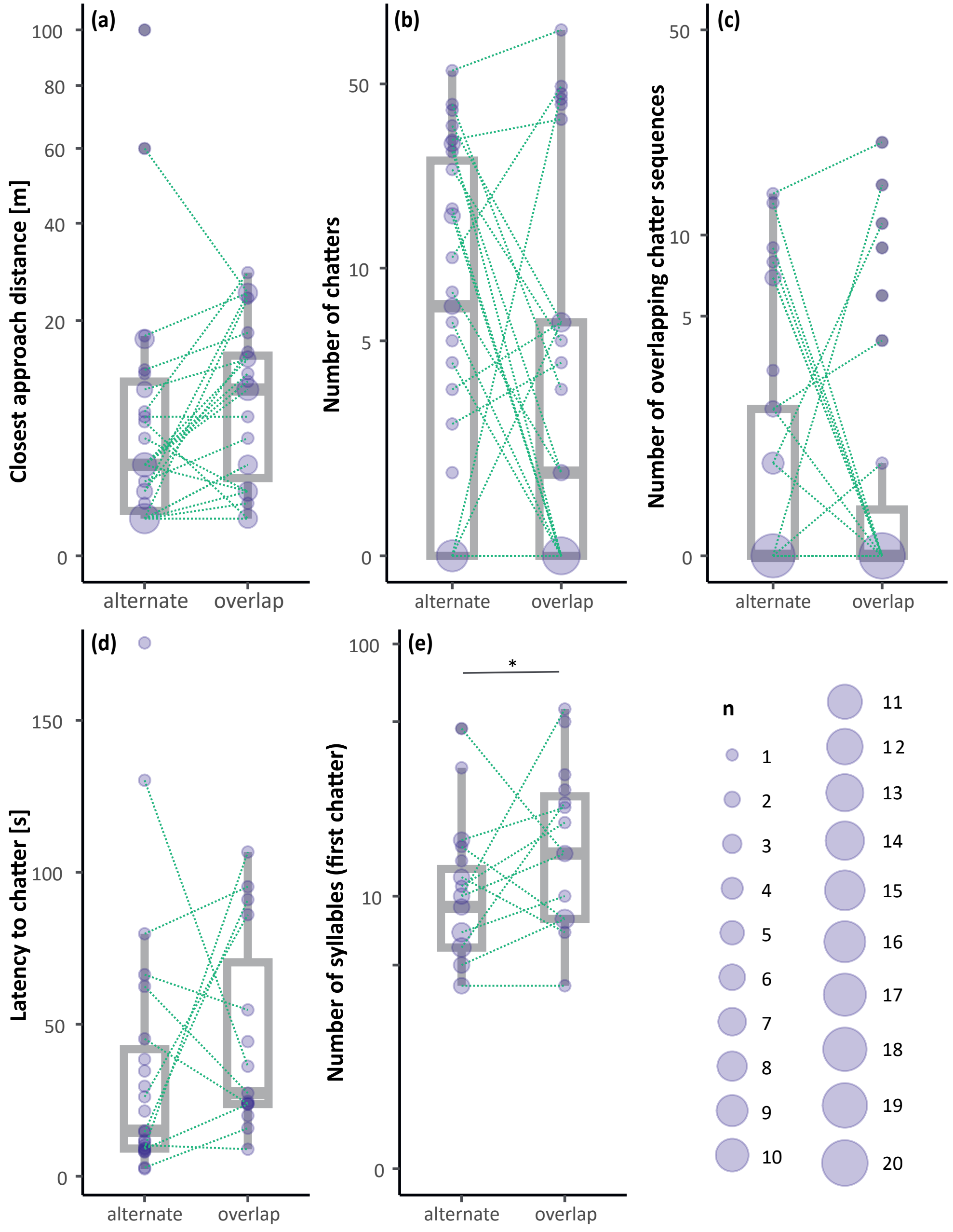


Figure 4.2. Magpie responses to playbacks of alternating and overlapping chatter calls. Boxplots per playback type display interquartile range (box), median (bold line) and the range of the data within 1.5 times the interquartile range (whiskers), and points for each trial (point size reflects the number of overlapping points). Trials at the same playback site are connected with a dotted line. The y-axis in (a) is square-root transformed and y-axes in (b), (c) and (e) are log transformed. Note that in (d) and (e) only those trials are included in which the birds chattered in response to playback. Horizontal line indicates the significant comparison between playback types (* $P < 0.01$).

Table 4.1. Results of all models testing the response of magpies to alternating or overlapping chatter playbacks.

	a) Closest approach distance			**b) Number of all chatters**			**c) Number of overlapping chatter sequences**			**d) Latency to chatter**			**e) Syllable number of the first chatter**		
	estimate (SD)	**test value**	***P***	**estimate (SD)**	**test value**	***P***	**estimate (SD)**	**test value**	***P***	**estimate (SD)**	**test value**	***P***	**estimate (SD)**	**test value**	***P***
Intercept (alternating)	2.45 (0.59)			2.13 (0.65)			-0.01 (0.93)			57.93 (19.37)			2.62 (0.35)		
Playback type (overlapping)	0.65 (0.34)	$\chi^2_1 = 3.68$	0.055	-0.55 (0.34)	$\chi^2_1 = 2.66$	0.10	-0.62 (0.51)	$\chi^2_1 = 1.45$	0.23	8.23 (12.21)	$\chi^2_1 = 0.45$	0.50	0.46 (0.22)	$\chi^2_1 = 4.62$	**0.032**
Trial sequence	0.01 (0.34)	$\chi^2_1 = 0.001$	0.98	0.24 (0.33)	$\chi^2_1 = 0.54$	0.46	0.07 (0.47)	$\chi^2_1 = 0.02$	0.89	-15.89 (11.97)	$\chi^2_1 = 1.76$	0.18	-0.13 (0.21)	$\chi^2_1 = 0.36$	0.55
Number of magpies	-	-	-	-0.01 (0.18)	$\chi^2_1 = 0.001$	0.98	0.26 (0.23)	$\chi^2_1 = 1.23$	0.27	-	-	-	-	-	-
Playback site (random effect)	Var (SD) = 2.18 (1.48), Residual Var (SD) = 1.37 (1.17)			Var (SD) = 0.64 (0.80)			Var (SD) = 0.65 (0.81)			Var (SD) = 140.1 (11.84), Residual Var (SD) = 1278.2 (35.75)			Var (SD) = 0.15 (0.38)		

Chapter 4

(4.5) Discussion

Since Eurasian magpie pair members often overlap each other's chatter calls during conspecific territorial intrusions, we hypothesised that such call overlapping would signal higher joint coordination, strength or motivation. Consequently, we predicted that playback of overlapping chatter calls would elicit a stronger response by a resident pair than playback of alternating chatter calls. Our results indeed show that magpies responded significantly differently to the two playback types. The first chatter call was significantly longer in response to overlapping chatter playback than in response to alternating chatter playback. However, contrary to our predictions, magpies tended to respond more cautiously to the playback of overlapping chatters by approaching marginally non-significantly less closely than they did to the playback of alternating chatters. None of the other vocal measures differed significantly between responses to overlapping and alternating chatter playback.

In terms of the duration of the first chatter call, magpies in our study discriminated between alternating and overlapping playbacks. These findings expand on our previous study showing that chattering towards conspecific intruders and ground predators can vary greatly in duration, from three to 124 syllables (Chapter 2). In predation contexts, longer mobbing calls may relate to a greater urgency or a more dangerous predator (see also Chapter 5), or are related to a more intense mobbing response (Leavesley & Magrath 2005; Templeton *et al.* 2005; Yorzinski & Vehrencamp 2009; Kalb *et al.* 2019), though also the opposite has been found (Carlson *et al.* 2020). In territorial defence contexts, the duration of short, harsh song elements (similar to the magpie chatter) was related to higher aggressiveness in barn swallows (*Hirundo rustica;* Galeotti *et al.* 1997). A longer first chatter may thus indicate an initially higher arousal, reflecting a stronger or more aggressive response (see also Chapter 5). In terms of the number of chatter calls or overlapping chatter sequences, however, magpies did not vary their response significantly between playback types, although higher call or song rates are commonly associated with more urgent threats, greater arousal and higher aggressiveness (Vehrencamp 2000; Leavesley & Magrath 2005; Shah *et al.* 2015). Our finding that the duration of the first chatter call did not correlate with any other measure of response (Table 4.A-2), suggests that the initial chatter reflects an initial assessment of the situation and has a different signal value than subsequent behaviour.

In contrast to the longer initial chatter to overlapping playback, the tendency to approach the alternating playback more closely suggests a higher complexity of how the timing of chatters calls is perceived. The closest approach distance is a typical measure for aggressiveness of a caller and indicates the strength or motivation of an intruder (Searcy *et al.* 2006; Araya-Ajoy & Dingemanse 2014; Diniz *et al.* 2021; Zapata & McEntee 2023).

Magpies tended to approach the loudspeaker more closely in trials with alternating chatter playback, though this was marginally non-significant. Contrary to our predictions and after their initial response, this nevertheless could suggest that magpies may have perceived alternating chatters as more threatening than the overlapping chatters.

Our seemingly contrasting findings in vocal responses versus approach behaviour are interesting in themselves. They may reflect different response strategies and uncertainty on how to interpret the different timings of the chatter calls without having additional contextual information. Chatter calls are very loud and thus a long-distance signal. Even before arriving closely at the scene (which differed between trials) and having been able to fully assess the contest, magpies may already have chattered in response to the playback. Hence, magpies in many trials may have integrated more information over time and may thus have interpreted the alternating, and effectively longer, chatter as a greater threat (see also Chapter 5). Apart from that, vocal and approach responses may not necessarily be correlated. In great tits (*Parus major*), for example, birds that sing much approach, on average, less closely and are less likely to attack a simulated intruder, likewise suggesting that they reflect different axes of response (Amy *et al.* 2010; Araya-Ajoy & Dingemanse 2014; Snijders *et al.* 2015). Also rufous horneros (*Furnarius rufus*) approached loudspeakers less during playback of coordinated duets compared to uncoordinated duets and nonoverlapping solo songs, but, unlike the magpies in our study, they did produce more highly coordinated duets in response to the coordinated duet playback (Diniz *et al.* 2021). Further research is thus needed to fully understand how magpies signal strength and aggressiveness in long-range signals and close interactions.

While we found that magpies discriminated between alternating and overlapping chatter playbacks, they may have attended to a variety of parameters that differed between our two playback types. By using the same number of chatter calls for both playback types, the playback types additionally differed in the total duration of chattering and the overall silence between chatters. This 'percentage performance time' (Poesel et al. , Kunc et al., Klump) is often used by individual signallers as measure of arousal or aggressiveness. Pairs that alternate their signals in time would in such a situation increase their signal time over those that overlap. Along this line, the effectively leader-follower 'duet' of the alternating chatters may have been perceived as a more coordinated chatter, given that the onset of the timing of the second chatter might have been more clearly detectable compared to the overlapping scenario. Hence, more detailed studies are required to disentangle which parameters of the alternating chatter playback elicit a seemingly (marginally non-significant) stronger response (i.e. closer approach) of magpies and how magpies can vary the signal value by coordinating their chatter behaviour.

(4.5.1) Conclusion

In this study, we tested if overlapping the partner’s alarm calls would enhance the joint signal strength over alternating chatter calling in territorial defence in Eurasian magpies, predicting that this would elicit a stronger response of territory holders. In line with our prediction, magpies emitted a significantly longer first chatter towards overlapping playbacks on the one hand. On the other hand, however, magpies tended to approach loudspeakers more closely in alternating playback trials. These results show that it matters how pairs coordinate their alarm calls. Thus, pairs may vary the combined signal by adjusting the timing of alarm calls in possibly a similar way as duetting songbirds do. Yet, it remains unresolved which exact parameters of the joint signal matter and alter the response, and in which ways strength, quality and aggressiveness are signalled in pair calling by magpies. Magpie chatter calls are a fascinating system not only because of the coordinated territorial defence of pairs but also because the same calls are used in territorial defence and anti-predator defence (Chapter 2). Investigations of a greater variety of species is needed to better understand the function and evolution of coordination among pair members in territory defence and predator avoidance.

(4.6) Acknowledgements

We thank Tim van den Bosch and Judith de Veer for finding and mapping magpie nests in our study area. We thank Dori Lukkezen, Huiyi Li and Tim van den Bosch for recording magpie vocalisations that were used in this study.

(4.7) Appendix

Table 4.A-1. Model results testing the response, i.e., a) magpies presence (glmm with binomial family distribution and logit link function) and b) total duration of chatters (glmm with a tweedie family distribution and log link function) to alternating or overlapping chatter playbacks, displaying the model estimates and Anova type II test results of all fixed predictors, variance and standard deviations of the random effect of playback site and the number of trials in the model dataset.

	a) Magpies present			**b) Total chatter duration**		
	estimate (SD)	**test value**	***P***	**estimate (SD)**	**test value**	***P***
Intercept (alternating)	4.35 (1.65)			2.24 (0.89)		
Playback type (overlapping)	-0.74 (0.66)	$\chi^2_1 = 1.23$	0.27	-0.21 (0.44)	$\chi^2_1 = 0.23$	0.63
Trial sequence	-1.52 (0.75)	$\chi^2_1 = 4.12$	**0.042**	0.27 (0.43)	$\chi^2_1 = 0.40$	0.53
Number of magpies	-	-	-	-0.04 (0.23)	$\chi^2_1 = 0.03$	0.87
Playback site (random effect)	Var (SD) = 2.36 (1.54)			Var (SD) = 0.83 (0.91)		
Sample sizes	40 (alternating), 40 (overlapping)			31 (alternating), 27 (overlapping)		

Table 4.A-2. Correlations (Kendall's tau) between all response variables, giving the tau values and sample sizes of each correlation.

	Latency to chatter	**Number of chatters**	**Total chatter duration**	**Number of overl. chatter sequences**	**Number of syllables**	**Closest approach distance**
Latency to chatter	x	n = 36	n = 36	n = 36	n = 38	n = 36
Number of chatters	τ = -0.39	x	n = 58	n = 58	n = 36	n = 56
Total chatter duration	τ = -0.36	τ = 0.92	x	n = 58	n = 36	n = 56
Number of overl. chatter sequences	τ = -0.18	τ = 0.72	τ = 0.72	x	n = 36	n = 56
Number of syllables (first chatter)	τ = -0.1	τ = 0.22	τ = 0.34	τ = 0.18	x	n = 38
Closest approach distance	τ = 0.08	τ = -0.35	τ = -0.37	τ = -0.33	τ = -0.11	x

Ch. 5 | Duration of an alarm and territorial defence call alters receiver response in a corvid

Miriam Kuspiel, Arnout Lindeman, Marc Naguib, Sjouke A. Kingma

(5.1) Abstract

Animals use vocal signals to provide information about a wide range of contexts. Across contexts, there is often extensive variation in vocalisation types since the adaptive value of vocal signals depends on the appropriate perception and response of receivers. However, some species, such as Eurasian magpies (*Pica pica*), use general alarm calls across a range of different types of threats (such as different predator types and conspecific competitors). Such general calls often differ in duration or call rates, raising the question whether this variation encodes information about the treat, and whether receivers can infer the meaning of the signal and respond appropriately. Using playback of calls of varying length and rates, we here tested whether territorial magpies respond differently to the variation in duration and rate in the general 'chatter' alarm call. We show that magpies responded acoustically faster to chatters of longer duration. Since magpies emit short calls to potentially more dangerous predators, chattering later to shorter calls may reflect an initially hesitant and danger-indicating response. Alternatively, magpies might respond less strongly to shorter calls because those calls signal lower motivation or urgency by intruders against whom the territory owner should defend. Interestingly, magpies did, overall, not approach the loudspeaker closer in response to longer calls and the total chatter response did not differ either. This suggests that after the initial response, the persistence of the signal over time as well as visual cues reflecting the level or type of danger become essential for more differentiated decisions to respond. Taken together, our results show that receivers can perceive and respond differentially to variation in alarm call duration in magpies.

KEYWORDS: Communication, alarm call, graded variation, call intensity, territorial intrusion.

(5.2) Introduction

Many animals use conspicuous vocal signals in territorial contexts as well as in predator-induced situations (Bradbury & Vehrencamp 2011). These signals often vary in structure and usage within and across contexts and can carry information about the signaller or the context (Vehrencamp 2000; Seyfarth & Cheney 2010; Suzuki 2016).

In threatening contexts, animals often use alarm calls to warn others or to mob the threat by calling and approaching (Leavesley & Magrath 2005; Smith 2017). Especially species with distinct responses to different predator types use predator-specific call types that carry information about the type of threat and allow receivers to take appropriate action (Macedonia & Evans 1993; Gill & Bierema 2013; Townsend & Manser 2013). Many alarm calls, however, are produced across a range of contexts, for example for predators hunting on the ground and agonistic interactions with conspecifics (Wheeler & Fischer 2012; Dezecache & Berthet 2018; Fichtel 2020). It has been suggested that the meaning of such "general alarm calls" may be encoded in relatively subtle variation in the call structure and usage, even though the context and appropriate response of receivers may substantially differ (Fichtel 2020).

Graded variation in alarm calls, either in the spectro-temporal call structure or the timing or rate of calls, has been associated with internal states of callers and may thus reflect the level of arousal or stress (Morton 1977; Schrader & Todt 1998; Fichtel & Hammerschmidt 2002; Schamberg *et al.* 2018). As such, longer calls reflect higher arousal and thus high urgency in some species (Yorzinski & Vehrencamp 2009; Seoraj-Pillai & Malan 2014; Szipl *et al.* 2017). Across many taxa, also higher rates of vocalisations have been shown to reflect high arousal or aggression, in predatory contexts and territorial conflicts (Smith 1977; Schrader & Todt 1998; Blumstein 1999b; Collins 2004; Suzuki 2016). For instance, more elements in their multi-element alarm call of white-browed scrubwrens (*Sericornis frontalis*) causes conspecifics to respond to threats faster (Leavesley & Magrath 2005). Likewise, black-capped chickadees (*Poecile atricapillus*) use "gargle" type calls and increased rates of song to communicate a warning of attack to competitors (Baker *et al.* 2012). Interestingly, if variation in calling rate or duration signals arousal or context, and receivers of the call can interpret this – potentially subtle - variation, this would explain also why the same, general, call types are often used across contexts in some species.

The alarm calls of Eurasian magpies (*Pica pica*; hereafter: magpie) are such an example, as their chatter calls are used over a wide range of contexts (Chapter 2). This raises questions of whether the calls are 'merely' used as a general alert call, or whether variation in the calls carries additional information and thus elicits a different response by receivers (Chapter 2).

The chatter call of the magpie is a loud trill of distinct syllables and a broad frequency, and is used while encountering predators as well as when competing with conspecifics (Birkhead 1991; Chapter 2). Although the same call type is used across different contexts, our previous studies show that the call duration and rate vary (Chapters 2 to 4). When encountering conspecifics or predators on the ground magpies use high rates of long chatter calls, whereas agile aerial predators (that are presumably more dangerous) invoke fewer and shorter calls (Chapter 2). This may suggest that long alarm calls function as a signal of strength or arousal when repelling intruders or predators, whereas short calls may reflect a high-danger alert call where a more cautious response by receivers is adaptive. However, it is still unknown whether or not receivers can interpret such variation in the call duration or rate, and whether this subsequently indeed affects the responses by conspecifics.

Here, we tested if magpies respond differently to playback of chatter alarm calls that differ in duration and rate. We exposed territorial magpies to playback of either (a) a series of long chatter calls, (b) a series of short chatters at high rate with, in total, the same time chattering as for the long chatters, and (c) a series of short chatter calls played at the same rate as the long chatter, and thus effectively less time chattering. If receivers perceive such variation and respond accordingly, we expect the strongest response to the playback of long chatters as used specifically in ground predator- and conspecific-induced contexts. We also expected a strong, yet weaker response to the high call rate playback, and overall a weaker and more cautious response to the low rate of short chatters.

(5.3) Methods

We conducted playback experiments on magpies from 19th August to 16th September 2022 in and around Wageningen, Ede, and Veenendaal, in the Netherlands (N52° 00.788' E5° 36.988'). The playbacks were conducted on 77 territorial magpie pairs, presumed to be on their all-purpose territories.

(5.3.1) Call recordings and playback preparation

The chatter calls for the playbacks (see below) were recorded in autumn 2021 and early summer 2022 using a Sennheiser ME66/K6 directional microphone connected to a TASCAM DR-100 digital audio recorder (sampling rate 44 kHz) in our study area. Chatter calls were elicited by placing a stuffed taxidermic model of a conspecific near focal magpies. In total, chatter calls from thirteen separate locations (with a minimum distance of 200 m between locations) were used in the playback experiments (3-18 chatters per location). We selected chatter calls with a minimum of 10 syllables, that were of decent quality and without disruptive background noise. Selected chatter calls were assorted to one of three playback types: long chatters, short chatters at a low rate, or short chatters at a high rate (Table 5.1, Figure 5.1). We assorted chatters from the same location equally over all playback types with the limitation that only chatters of minimally 13 syllables could be assorted to the long playback type. In Audacity® v3.1.3 (Audacity Team, 2023), we cut each selected chatter call to the appropriate number of syllables, high-pass filtered it at 300 Hz with 48 dB roll-off, normalised it to an amplitude of -1 dB, and applied fade-in and fade-out effects up to the start of the first syllable and from end of last syllable (over 0.02 to 0.03 s). For a playback file, we repeated the same call the corresponding number of times (Table 5.1) within one minute with irregular intervals between calls (Figure 5.1), and saved it as wav file. To create the final order for chronological use, all playback files were randomly ordered within sets of three (one of each experimental group) to ensure equal numbers of trials per playback type. The playbacks were played at a peak sound pressure level between 88.6 and 94.4 dB SPL (measured from a 1 kHz sine wave normalized to the same peak amplitude as the chatter calls with a Voltcraft SL300 SPL meter (A-weighted, fast) at one meter distance to the loudspeaker on different days outside with full or low battery power of the loudspeakers).

Table 5.1. Characteristics of the three playback types created and used in this study.

	long	short - low rate	short - high rate
Syllables per call	13 - 14	5 - 6	5 - 6
Number of calls	17 - 18	16 - 18	40 - 44
Total number of syllables	233 - 240	90 - 96	220 - 240

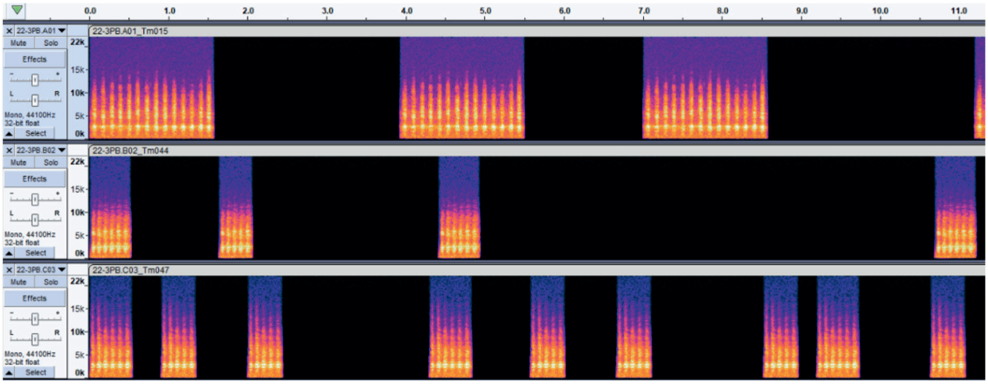


Figure 5.1. Spectrograms of the first 11 seconds of exemplary playback files of long chatters (top), short chatters at a low rate (middle) and short chatters at a high rate (bottom) with the frequency in Hz on the y-axis, the time on the x axis (top) and colours indicating the sound pressure level of a given frequency at a given time, ranging from black over purple to bright yellow (normalised to -1 dB).

(5.3.2) Playback experiments

In total, we conducted 77 playback trials on magpie pairs between 07h00 and 12h00. Once we located a magpie pair, we placed the loudspeaker (UE Boom 2) in a bush or tree 50 to 150 cm aboveground and 0.5 (when a bird was high in a tree) to 65 m from the closest magpie (measured in the horizontal plane, depending on the availability of suitable locations in the area and where magpies moved to until we started the playback). The observer moved minimally 13 meters away from the loudspeaker (depending on the openness of the trial area). The loudspeaker was connected via Bluetooth to the observer's phone to control the playback. If the birds remained within estimated hearing range during setup, the playback track was started and all birds within the target area were observed and recorded for three minutes (1 min playback + 2 min post-playback). The behavioural response of the birds to the playback was recorded using two Sennheiser ME66/K6 microphones connected to the two channels of a TASCAM DR-100 recorder. One microphone was pointed at the magpies and the other microphone was used to for voice comments on the number of magpies, their start locations and movements. Post-trial, we measured the distances from the speaker to all landing positions of the birds using range meters. Distances closer than ten meters to the loudspeaker were measured in detail, using, for example, specific branch locations and points on the ground. Farther positions were measured using reference points, such as tree trunks or roofs, because the magpies were not always clearly visible in more distant locations. If the position of a bird was unclear at any point, the average distance between the two most likely positions was taken. We maintained a minimum distance of 200 m between trial sites to minimise the probability of the same pairs being measured again (based on neighbour-nest distances in European magpie studies with similarly high breeding densities; Birkhead 1991; Lehmann *et al.* 2005). We further maintained 400 m distance from the location where the corresponding playback call was initially recorded to avoid playing back chatter calls of the focal individuals or their close neighbours.

(5.3.3) Acoustic analyses

In Audacity® v3.1.3 recording and editing software (Audacity Team 2023), we identified chatter calls by listening and inspecting spectrograms. We marked the trial start and all chatter calls of focal magpies with labels. Labels were set over the entire call for single chatters and over the entire overlapping chatter sequences for overlapping chatters of focal magpies. Chatter labels contained the number of chatter syllables (for clear, single chatter calls), whereas chatters in overlapping sequences were labelled as "all" (entire sequence), or "second", "third", etc., for the position in a sequence. When a call was faint or remarked by the observer as "distant" (i.e. from beyond the trial area), it was labelled accordingly excluded from further analyses.

The labels were exported as text files including the start and end time of the labels. Those files were merged with the noted trial information in R v4.3.2 (R Core Team 2022) to quantify per trial (25 of long chatter playback and 26 each of short chatter playbacks at high and low rates): a) the closest approach distance of magpies during a trial, b) the number of all chatters (including overlapping chatters) and the total duration of single chatters and chatter sequences. For those trials in which magpies chattered (18 with long chatter playback and 20 each with short chatter playback at both high and low rates), we further extracted c) the latency from the start of the playback to the first chatter call, and d) the number of syllables of the first chatter call (which was unclear for the first chatter in one trial).

(5.3.4) Statistical analysis

We tested if magpie responses (see a-d below) differed between playback types in R v4.3.2 (R Core Team 2022) with generalised linear mixed models with the package glmmTMB v1.1.8 (Brooks *et al.* 2017). In all models, we included the square-root transformed start distance of magpies from the loudspeaker as scaled fixed variable to control for potential differences in the perceived urgency of a threat. We square-root transformed both the distance of the closest magpie at start and the closest approach distance of any magpie in a trial to give greater weight to differences at close distances (e.g. one versus two meters) than at far distances (e.g. 31 versus 32 meters). Start distance did not vary significantly between playback types (Anova type II Chi-square test of playback type as fixed predictor for the square-root transformed start distance: $X^2_2 = 3.08$, $P = 0.21$). We chose the appropriate family link function of the model based on the type of data (count or continuous) and for which Dharma v.0.4.6 plots best fit model assumptions (e.g. no overdispersion). Collinearity of predictors, tested with the performance package v0.10.5 (Lüdecke *et al.* 2021) was low for all models (VIF < 2.5; Johnston et al., 2018).

(a) *Closest approach distance*. For the effect of playback type on the square-root transformed closest approach distance, we used a tweedie family distribution with a log link function.

(b*) Number of chatters*. For the effect of playback type on the number of all chatters in a trial, we used a negative binomial 1 family distribution with a log link function and included the number of magpies in a trial as fixed factor to account for multiple potential callers.

(c) *Latency to chatter*. For those trials in which magpies chattered, we tested for an effect of playback type on the latency to emit the first chatter with a tweedie family distribution with a log link function.

(d) *Number of syllables (first chatter)*. For those trials in which magpies chattered, we further tested for an effect of playback type on the number of syllables of the first chatter in a trial with a generalised poisson family distribution with a log link function.

We performed Anova type II chi-square tests on all models to obtain test statistics of each fixed factor. We further conducted post-hoc pairwise comparisons of estimated marginal (least-square) means between the three playback types with the emmeans package v1.8.2 (Lenth 2022).

(5.4) Results

Over all trials, magpies did not differ in their closest approach distance (Figure 5.2.a, Table 5.2.a) or the number of chatters (Figure 5.2.b, Table 5.2.b) in response to the different playback types. However, those birds that chattered in response to playback, chattered, on average, earlier to long chatter playbacks: the latency to emit the first chatter (Figure 5.2.c, Table 5.2.c) was significantly shorter in response to long chatter playback than to playback of short chatters at a low rate (estimated marginal mean ± SE: 15.5 ± 3.2 s (long), 38.2 ± 7.5 s (short – low rate)). The latency to chatter in trials with playback of short chatters at a high rate was also, although not significantly, on average 9.5 seconds later than to playback of long chatters, yet, again not significantly, on average 13.2 seconds later than to playback of short chatters at a low rate (estimated marginal mean ± SE: 25.0 ± 4.9 s). Still, within those trials with long chatter playbacks, magpies appear to either chatter very early or later, leading to a seemingly almost bimodal distribution (Figure 5.2.c). The duration of the first chatter call (measured as the number of syllables) did not significantly differ between playback types (Figure 5.2.d, Table 5.2.d). In addition, regardless of playback type, magpies approached significantly closer and chattered more when the start distance between the loudspeaker and magpies was shorter (Table 5.2).

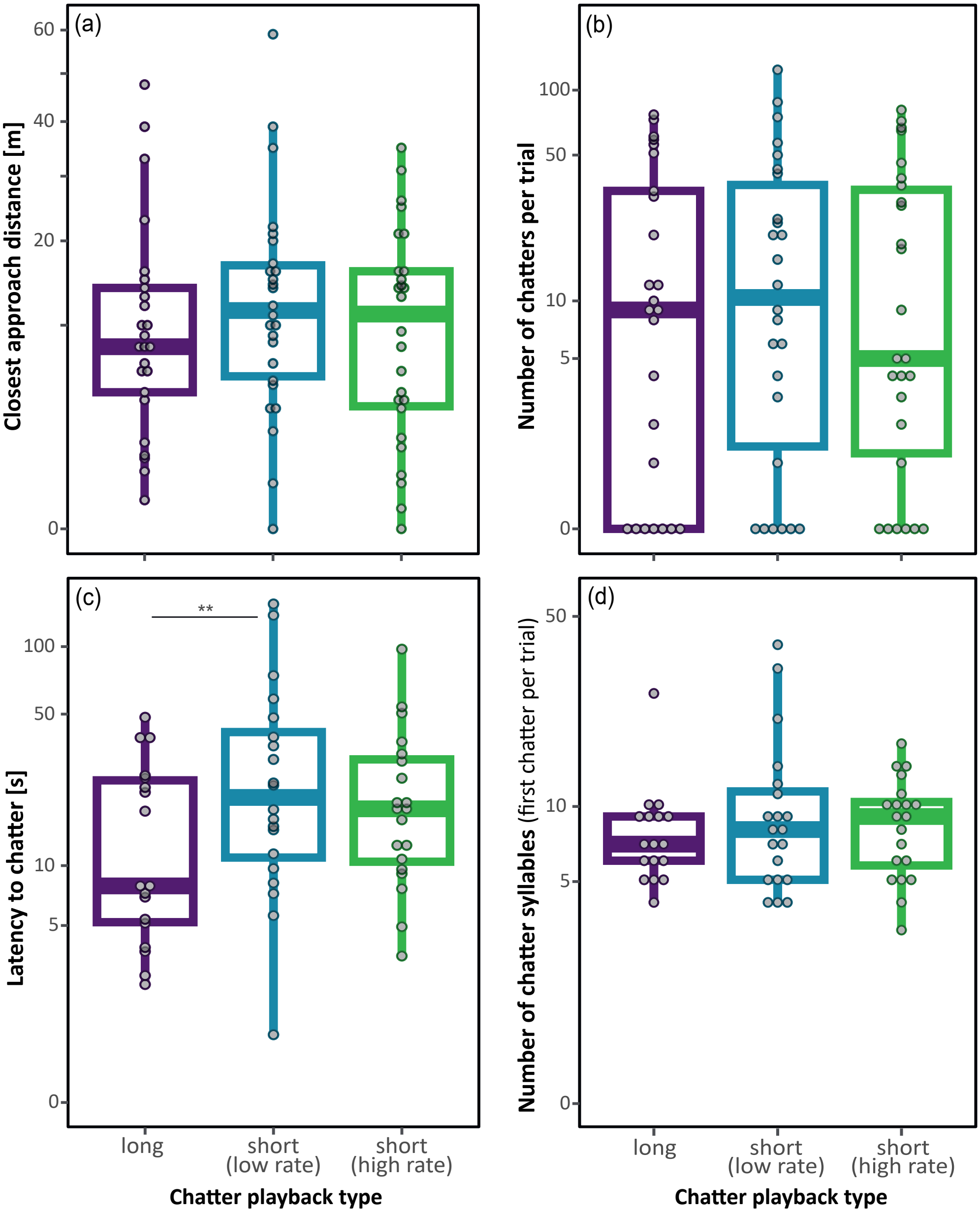


Figure 5.2. Magpie responses (y-axis) to the three chatter playback types (x-axis). Data points per trial are given as circles and boxplots show the median (bold line), inter-quartile range (box) and 1.5 times the interquartile range (whiskers) per playback type. The y-axis of (a) is square-root transformed and the y-axes of all other plots (b-d) are log transformed. Horizontal line indicates the significant pairwise comparison (***P* < 0.01).

Table 5.2. Model results for how magpies responded (variables a to d) to the three different playback types. Given are the estimates and Chi-square tests of all fixed factors in the model and the estimates and z ratios of the pairwise comparisons between playback types. Significant effects ($P < 0.05$) are indicated in bold.

	a) Closest approach distance			**b) Number of chatters per trial**			**c) Latency to chatter**			**d) Number of chatter syllables (first chatter)**		
	estimate (SE)	**test values**	***P***	**estimate (SE)**	**test values**	***P***	**estimate (SE)**	**test values**	***P***	**estimate (SE)**	**test values**	***P***
(intercept)	1.05 (0.11)			3.03 (0.26)			2.72 (0.21)			2.11 (0.14)		
Playback type		$\chi^2_2 = 1.87$	0.39		$\chi^2_2 = 0.05$	0.97		$\chi^2_2 = 9.93$	**0.007**		$\chi^2_2 = 1.35$	0.51
… long - short (low rate)	-0.19 (0.14)	$z = -1.34$	0.18	-0.04 (0.3)	$z = -0.15$	0.88	-0.9 (0.29)	$z = -3.15$	**0.002**	-0.22 (0.19)	$z = -1.16$	0.25
… long - short (high rate)	-0.07 (0.15)	$z = -0.47$	0.64	0.02 (0.3)	$z = 0.07$	0.95	-0.48 (0.28)	$z = -1.68$	0.092	-0.13 (0.18)	$z = -0.7$	0.48
… short (low rate) - short (high rate)	0.12 (0.14)	$z = 0.87$	0.39	0.07 (0.29)	$z = 0.22$	0.82	0.42 (0.27)	$z = 1.54$	0.12	0.09 (0.17)	$z = 0.52$	0.60
sqrt(closest start distance)	0.18 (0.06)	$\chi^2_1 = 10.12$	**0.002**	-0.32 (0.13)	$\chi^2_1 = 6.01$	**0.014**	0.19 (0.12)	$\chi^2_1 = 2.63$	0.11	0.1 (0.07)	$\chi^2_1 = 1.95$	0.16
number of magpies		-		0.11 (0.14)	$\chi^2_1 = 0.67$	0.41		-			-	

(5.5) Discussion

In this study on magpies, we tested the prediction that receivers of alarm calls respond differentially to calls of varying duration and rate, assuming that these features reflect degrees of urgency and thus elicit different responses. Magpies did not differ in their closest approach distance and the total number of chatters in response to the different playback types. Yet, those magpies that chattered in response to playback, chattered earlier in response to longer chatters, particularly compared to when short calls were played at a slow rate. This result suggests that variation in duration in the general magpie alarm call provides information on the urgency of the context. There are different potential reasons for why a longer chatter may elicit a faster response, as we discuss here.

First, magpies may have perceived long chatters as a more threatening competitor intruding their territory since chatters are frequently produced during simulated territorial intrusions (Chapter 2). Magpies also responded similarly to the playback of chatters in this experiment as they did to taxidermic models of a conspecific intruder (Chapter 2). If longer chatters signal the strength, aggressiveness or motivation of the intruder (Galeotti *et al.* 1997; Schwartz *et al.* 2002; Ansell *et al.* 2020), playback of long chatters would probably be perceived as greater or more urgent threat that requires a faster response (Manser *et al.* 2001; McLachlan & Magrath 2020). This may explain why we observed a shorter latency to chatter to long chatter playback. A fast response may then be used to repel competitors (Bradbury & Vehrencamp 2011). However, in contrast to our predictions, territory owners did overall not respond stronger, i.e. with more chatters, a longer first chatter, or, importantly with a closer approach, to chatters of longer duration. The large variation in habitat structure and start distance may have masked some of the variation, though it did not differ significantly between treatments. Still, we would have expected stronger responses to signals that are apparently perceived as more urgent (i.e. elicited an earlier chatter response), which was not the case. The latency to respond appears to reflect best the initial assessment of the urgency of a signal (McLachlan & Magrath 2020) and suggests that longer chatters signal higher urgency. In other words, territory owners can be expected to signal faster to motivated competitors that the territory is occupied by calling back sooner, indicating their alertness and readiness to respond. Interestingly, there was substantial variation in how fast magpies responded and the response time distribution to long chatters seemed rather bimodal. This might indicate that the response time depends on the quality of individuals relative to that of the intruder, where only individuals that consider themselves stronger respond fast (Collins 2004). To test this idea, future studies would be worthwhile to test whether alarm call duration may indeed reflect the quality or strength of individual magpies.

Second, magpies may perceive the differences in chatters as a reference to a certain type of predator, which may explain why we found a difference in response latency. In our previous study (Chapter 2), we showed that relatively long chatter calls are emitted to not only to conspecific intruders but also to less dangerous ground predators, whereas chatters to agile and presumably more dangerous aerial predators were, on average, shorter. This may explain a somewhat more cautious initial response towards the playback of short chatter calls. However, we would further expect magpies to be consistently more cautious throughout the trial if a short chatter indicates the presence of a dangerous predator, but we did not find this. The overall approach distances, chatter rates and characteristics did not vary across the different call type playbacks. This suggests that magpies may interpret the chatters of territory intruders as a sign of presence of intruders rather than as reference to predator type, at least as soon as they found no other cues for the presence of a predator (Fichtel 2020). Moreover, magpies cease to alarm call very quickly in situations where aerial predators are encountered (Chapter 2), perhaps because they require an immediate response (McLachlan & Magrath 2020). When signals are emitted for longer as in our experiments (even though each call is of short duration), the interpretation of such signals might change (Fischer 2013; Fichtel 2020). While the initial chatter may be interpreted as a warning for a predator, the continued chattering may be rather perceived as a threat in itself, i.e. an intruder in the territory. This may explain why we only found a difference in initial chatter latency, and not in other responses measured over the entire trial. Experiments with very brief playbacks where only few chatter calls are emitted (as in Naguib *et al.* 2000) would be worthwhile to test this idea, to prevent that information gathered throughout a longer playback changes an initial assessment.

In contrast to our finding that longer calls elicited a stronger initial response, our experiment does not indicate that magpies perceive a higher rate of calls as stronger threat. Although they chattered slightly later to short calls at a low-rate than to chatter calls at a high-rate short calls, this difference was not significant, and other responses (call rates, approach distance) did not differ either. This suggests that the higher call rate used in our experiments does not encode a higher degree of urgency, similarly as in other species such as for example the common cuckoo (*Cuculus canorus;* Elek *et al.* 2021) yet in contrast to multiple other studies (Smith 1977; Collins 2004; Suzuki 2016; Schamberg *et al.* 2018). Alternatively, already the low rate of short chatter calls may have been perceived as a high threat (for example of a territorial intruder) so that magpies responded equally strong overall (as we explain above for chatter call duration). Nonetheless, we conclude that call rate, at least at the rate and duration as used in our experiment, unlikely carries information of danger or urgency in magpies.

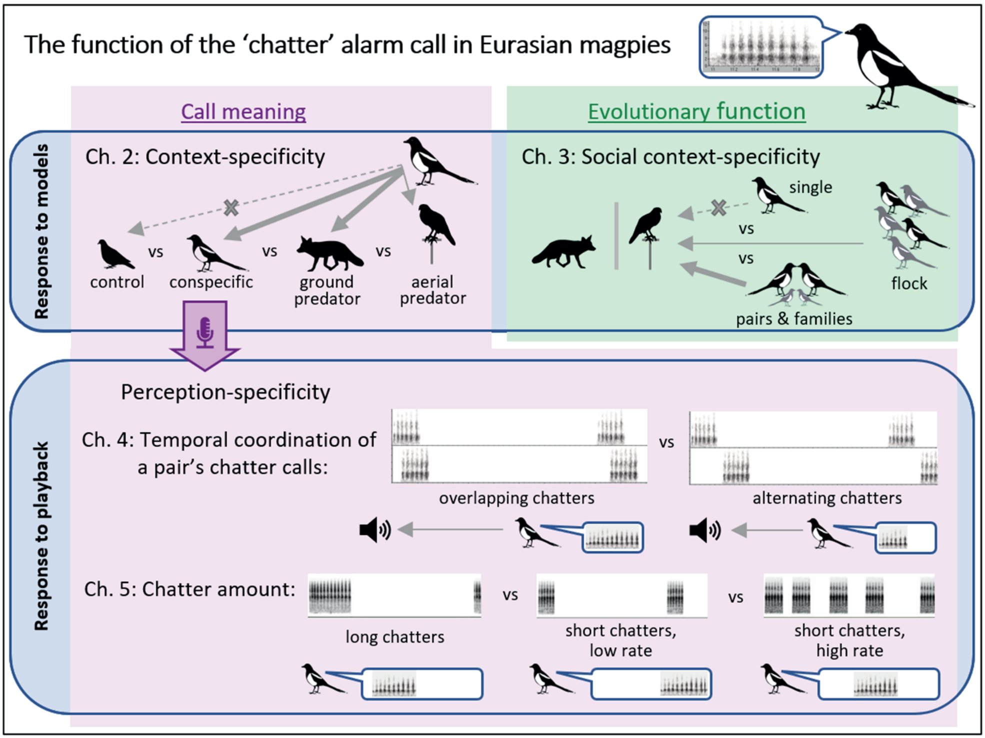
Taken together, our finding that magpies responded faster to longer alarm calls shows that call duration carries relevant information and suggests that longer calls signal higher urgency, and more so than high rates of short calls. The lack of subsequent response differences towards the different playback types suggests that the persistence of calls over time or the presence of visual cues of the actual treat type are relevant for interpreting call information. These context-specific definitions of call meanings highlight the complexity of the function of signals, where the interpretation of the signal depends on a variety of factors than purely the type of a call.

(5.6) Acknowledgements

We thank Dori Lukkezen, Huiyi Li and Tim van den Bosch for recording magpie vocalisations that were used in this study.

Ch. 6 | General discussion: The function of alarm calls and their variation

Alarm calls have long been subject to extensive studies in birds and mammals. Because the context and function of alarm calls seem to be easily identifiable, they are an intriguing subject to understand the mechanisms of encoding information and even to compare vocal signalling in humans and non-human signalling (Marler *et al.* 1992; Rendall *et al.* 2009; Fedurek & Slocombe 2011; Suzuki 2016). Additionally, evolutionary biologists are intrigued by the question why individuals should alarm call and which benefits may favour the evolution of alarm calls (Charnov & Krebs 1975; Curio 1978; Blumstein 2007; Zuberbühler 2009; Carlson & Griesser 2022). I integrated several of the open questions on the proximate (i.e. mechanistic) and ultimate (i.e. evolutionary) function of alarm calls in my thesis to better understand the meaning and function of an alarm call in a corvid.



6.1. Schematic overview of my PhD thesis, in which I tested approach, threatening and vocal responses to taxidermic models representing different threat types in Chapters 2 and 3, and tested approach and vocal responses to playbacks of different chatter variations in Chapters 4 and 5.

(6.1) The function of calls: Inferring meaning and evolutionary drivers from the contexts of call production

In Chapters 2 and 3 of my thesis, I explored in which contexts chatter calls are produced to infer the likely function of those calls. I verified that chatter calls classify as a "general alarm call" which is produced not only across different predatory but also in agonistic contexts with conspecifics. In this sense, magpie chatter calls appear to bridge the fields of predator related alarm calling and conflict resolution among conspecifics. The different function of calls and benefits why magpies alarm call likely vary between those contexts, making it an intriguing system to understand how call meaning may be influenced by the context.

(6.1.1) When to alarm call to repel which threats

We observed that magpie pairs produce high rates of chatter calls towards both ground predator models and conspecific territorial intruders (Chapter 2 and 3). Together with closer approaches to the model and occasional other threatening displays, these chatters likely function as repellent towards the conspecific or ground predator. While many studies in the past focussed on assigning specificity to different predator types only, follow-up research discovered that even seemingly predator-specific calls are also produced in non-predatory contexts (Price *et al.* 2015; Dezecache & Berthet 2018; Fichtel 2020). Even the 'leopard' calls in vervet monkeys are also frequently produced in agonistic interactions with conspecifics (Price *et al.* 2015). In my research, I therefore compared also other behaviours accompanying the chatter calls in magpies. Even though magpies did not approach and attack the ground predator models with the same intensity as they did models of conspecifics, their behaviours did not significantly differ, and we observed similar approaches and attacks towards the fox models in some trials (Chapter 2). The high call rates and approaches to the ground predator are two characteristics of mobbing behaviour, and a major function of mobbing calls seems to be the repellence of the predator (Carlson & Griesser, 2022; Curio, 1978). The chatter calls in both predator and agonistic contexts thus seem to serve a similar function, i.e. repelling the threat. The loud and harsh sound of the chatter call as well as mobbing or aggressive calls in other bird and mammal species have been proposed to signal agonistic intend (Morton 1977; Ficken & Popp 1996; Owren & Rendall 2001). If most species would perceive a call like a magpie chatter call as threatening or annoyance, there would be no selective pressure to evolve different signals for deterring conspecifics and predators.

Our results from investigating the social environment in which mobbing occurs (Chapter 3) also indicate that chatter calls are used to repel ground predators. We found that mainly pairs and families during the breeding season intensely called at and approached the predator. At that time, repelling the predator would be beneficial to protect offspring which are at a high risk of being predated (Baeyens 1981a; Birkhead 1991). Even if offspring are still safely in the nest, they may benefit in the future if mobbing deters predators from hunting in the territory in the future (Curio 1978; Stewardson & Brett 2000; Crofoot 2013; Carlson & Griesser 2022). While evidence that mobbing deters a predator, long-lasting effects have been barely studied so far (Flasskamp 1994; Pavey & Smyth 1998; Clark 2005; Gursky 2006; Carlson & Griesser 2022). Even outside the breeding season, some magpie pairs emitted many chatters and closely approached the ground predator model, potentially to protect their partner or defend their territory against predators (Chapter 2). In contrast, single magpies and magpies in flocks hardly ever closely approached and called at ground predator models at high rates in our study. Thus, magpies seem to invest the time and energy of mobbing and potentially risk injuries or death (Crofoot 2013; Carlson & Griesser 2022) mainly to protect their partner and to keep predators out of the territory. Protection of offspring or kin indeed seems to be the most widespread function of mobbing a predator (Schwagmeyer 1980; Shedd 1982, 1983; Cully & Ligon 1986; Griesser & Ekman 2005; Blumstein 2007; Stephan & Zuberbühler 2021; Carlson & Griesser 2022). Hence, predator mobbing may have evolved as a form of extended parental care (Stephan & Zuberbühler 2021) but may be beneficial also in other social contexts in other species.

Mutualistic or group-related benefits may be gained from mobbing in groups. In a group, the risk to the individual would likely be diluted and a larger mobbing flock may be more likely to succeed in deterring the predator (Flasskamp 1994; Gursky 2006; Wheeler 2008). Groups may also contain kin from which to derive inclusive fitness benefits (Hamilton 1964; Maynard Smith 1965; Hoogland 1983; MacWhirter 1992; da Silva *et al.* 2002; Wheeler 2008). Group members may also provide group benefits through, for example, communal roosting, finding food, or protection from predators (Clutton-Brock 2002, 2009). However, if predators are mobbed for such benefits is hard to assess and has rarely been attempted (Cheney & Seyfarth 1985; Randall *et al.* 2000; Wheeler 2008; Teunissen *et al.* 2020). Some of the strongest evidence that predators are mobbed for group benefits could be found in a cooperatively breeding bird: Non-breeders that stay with their parents as helpers likely derive group benefits and are more likely to mob adult predators to protect group members than to mob nest predators, although nest predators pose a lower risk to mobbers (Teunissen *et al.* 2020). Magpies in flocks, however, were rarely observed to emit a high number of chatters and to closely approach the predator model (Chapter 3). Thus, neither kin selection nor group benefits seem to drive predator mobbing in magpie flocks.

Multiple aspects may explain why kin selection or group benefits do not appear to drive intense predator mobbing in magpies: The costs of mobbing may be too high, the benefits to gain from conspecifics may be too low, but also the benefit the conspecifics gain from a predator being mobbed may be too low (Klump & Shalter 1984). In contrast to young fledglings that are highly susceptible to predation, a vigilant adult magpie seems unlikely to be predated by a fox and thus has little to gain from the predator being mobbed (Buitron 1983; Birkhead 1991). Even older fledglings that are skilled in flying may not be at a high risk from foxes anymore, so the benefits from mobbing a fox might have been smaller in some trials with older fledglings present. This could explain why we observed great variation in mobbing intensity even within trials on families (Chapter 3). Supporting this idea, Buitron (1983) observed anti-predator responses to change from threatening and attacking predators to mostly vocalising with the age of fledglings. The benefits that magpies with good flying skills could gain from predators to be mobbed may thus be very low. Yet, the potential risk of being attacked by the ground predator during mobbing seems similarly low, especially if it is mobbed from an elevated position or by a larger flock. Still, mobbing a predator costs energy and time, which is traded off against, for example, foraging (Crofoot 2013). In sum, the benefits that flock members or also the partner may gain from a predator being mobbed, and thus the benefits the mobber may gain from protecting those conspecifics (see above), do not seem to overweigh the potential costs of predator mobbing in magpies. We would need to investigate mobbing intensity in a variety of species to understand if adult vulnerability to predation or what kind of potential benefits to be gained from protecting conspecifics may drive the evolution of mobbing outside the context of parental care.

Intense mobbing in our studies was largely restricted to ground predators while aerial predators were rarely mobbed (Chapters 2 and 3), which contrasts with studies blackbilled magpies and other birds (e.g., Buitron 1983; Hogstad 2005; Courter & Ritchison 2010; Dutour *et al.* 2016). Even magpie pairs during the breeding season emitted higher numbers of alarm calls (eight to 35) in only five of 13 aerial predator trials, and approached the raptor model to within ten meters in only one trial (Chapter 3). Either the costs of mobbing an aerial predator in contrast to a ground predator are higher or the offspring would benefit less from raptors being mobbed (see previous paragraph). The ground and aerial predator models that we used in our studies may not primarily hunt magpies but have been reported to predate on magpies and to occur in our study area, thus are likely to pose a threat to magpies (Buitron 1983; Birkhead 1991; Macdonald *et al.* 2017). Since magpies mobbed taxidermic models of ground predators and conspecifics and stayed further from taxidermic models of ground predators and control species (Chapter 2), it seems unlikely that magpies perceived the taxidermic models to be artificial and therefore did not mob them. Unlike a raptor capable of pursuing a prey in flight, however, a ground predator can be safely mobbed from an elevated perch. Magpies may thus mob ground predators because it is less risky to themselves. In line with this argument, our findings indicate that risky behaviours like landing on the ground close to the model and swooping over it were mostly avoided even in ground predator trials (Chapter 2). Several other studies also show that the risk to mobbers influences if or how different predator types are mobbed, with less risk-taking for more dangerous predators (Curio *et al.* 1983; Koboroff *et al.* 2013). I further argue that also mobbing a raptor would be beneficial. Even though the threat a perched sparrowhawk poses to adults and fledglings may be negligeable since raptors are most dangerous in flight and on sudden appearance (Cresswell 1994a; Petracca & Caine 2013), mobbing would likely result in the raptor leaving the area and thus seizing to be a threat (Buitron 1983; Pettifor 1990; Flasskamp 1994). Moreover, mobbing a predator could teach offspring about dangerous threats and thereby increase their future survival, which has been proposed for meerkats and to be important for juvenile Siberian jays (Curio 1978; Graw & Manser 2007; Haff & Magrath 2012; Griesser 2013). It thus seems that magpies integrate the risk to themselves in their decisions on which predators to mob, though this needs to be tested directly. It also remains unclear why other, even smaller birds, still mob highly dangerous raptors (Curio 1978; Randler & Vollmer 2013; da Cunha *et al.* 2017a), and if this is driven mainly by greater benefits derived from mobbing or lower costs to be paid, for example in larger flocks (da Cunha *et al.* 2017a; Carlson & Griesser 2022). My findings emphasize the importance to explore responses towards different predator types as well as the costs and benefits of these responses to fully understand when and why animals mob predators.

(6.1.2) When to alarm call to recruit conspecifics

The second proposed function of mobbing calls is to recruit conspecifics to the mobbing scene (Curio 1978; Carlson & Griesser 2022). Thereby, the risk to the individual mobber is diluted, the predator may be confused if called at and threatened from multiple sides, and a larger group may be more successful in repelling the threat (Caro 2005; Gursky 2006; da Cunha *et al.* 2017a). In fact, the definition of mobbing has partly been restricted to a greater number of individuals signalling at and approaching the predator together (Carlson & Griesser, 2022). Mobbing assemblies are known for various group-living mammals such as primates and meerkats (Graw & Manser 2007; Crofoot 2013; Rubow *et al.* 2017). Also in birds, large assemblies that often contain multiple bird species can be observed mobbing a predator together (Curio 1978; da Cunha *et al.* 2017a).

In magpies, some reports indicated that predators are mobbed by a group of more than the two parents during the breeding season (Buitron 1983; Birkhead 1991). Moreover, at the start of the breeding season, so-called “gatherings” have been described where not only a floating pair but even a flock intrudes on a territory to battle for the ownership (Birkhead 1991). In those cases, neighbouring pairs have been suggested to combat these flocks together (Birkhead 1991).

During my studies, I occasionally observed that other magpies, mostly the apparent neighbours, were attracted by the chatter calls in response to the magpie or ground predator models. While they may have joined in vocalising, they often stayed further away (at least 30 m), potentially at the boundary of the territory, and did not approach closer. In two cases where a neighbouring pair flew over the fox model or attempted to land nearby, they were immediately chased further away by the focal pair before the latter continued mobbing the fox model. Thus, recruiting neighbours seems to be rather a side effect of the clamour and not the intention of the callers (Fichtel & Manser 2010). This is surprising considering the probably greater success in repelling the predator with more mobbers (Gursky 2006; Carlson & Griesser 2022). It also stands in contrast to other bird species where neighbours help in mobbing nest predators or brood parasites (Krams *et al.* 2006; Welbergen & Davies 2008; Krama *et al.* 2012; Barrionuevo *et al.* 2019). Nevertheless, magpies appear to perceive the threat of territorial intruders equally great as the threat a predator poses. Still, magpie pairs usually defended their territory together. Chatters of pairs may therefore also function to recruit the partner and keep it engaged in mobbing the predator.

(6.1.3) When to alarm call to warn conspecifics

Even though aerial predator models in our studies were apparently not intensely mobbed, we still recorded a few chatter calls in many aerial predator trials (Chapter 2 and 3). The function of these calls produced in a non-mobbing context unlikely function as mobbing calls to repel the predator. Still, even emitting just one call could function as perception advertisement, aiming at de-motivating the predator from attacking and encouraging it to hunt elsewhere instead (Caro 1995; Shelley & Blumstein 2005; Huang & Caro 2023). If magpie chatters in aerial predator trials were mainly addressed at the predator, however, we would also have expected single magpies to emit such signals (Shelley & Blumstein 2005). However, single magpies rarely emitted a chatter call in aerial predator trials (Chapter 3). In contrast, magpies emitted a chatter call in over half of the aerial predator trials when other magpies were in the vicinity. In other species, such audience effects have been used to infer the intended receiver of a call (Gyger 1990; Marler & Evans 1996; Fichtel

& Manser 2010). Since male fowl (*Gallus gallus*) only emitted a call on a simulated approach of a raptor if conspecifics were in the vicinity and because this call elicited the appropriate anti-predator response in call receivers, the authors concluded that these calls function as alarm signal to conspecifics (Gyger *et al.* 1986; Karakashian *et al.* 1988). Similarly, magpie chatter calls in aerial predator contexts may well function as warning signal to conspecifics.

While we observed mobbing calls in only very specific social contexts, apparent warning signals (i.e. chatters in response to raptor models) were generally produced when other magpies were in the vicinity, i.e. in pairs, families and flocks (Chapter 3, although the calls in flocks may also be caused by the presence of more potential callers). In the previous section, I discussed that adults may not profit much from mobbing and repelling the predator as they are not likely to fall prey to a ground predator or perched raptor. However, an unvigilant magpie may still be caught by an ambush predator (Birkhead 1991). Even adult magpies may thus benefit from being warned about the presence of a predator. In addition, the costs for emitting just one or few brief warning signals are likely lower than the costs for intense mobbing behaviour (Crofoot 2013). Both the greater benefit to call receivers and the lower costs of calling may thus explain why we observed magpies to warn conspecifics in a greater variety of social contexts, which I discuss below.

First, pairs emitted few chatters (presumably warnings) in many trials with ground and aerial predators also outside the breeding season (Chapters 2 and 3). During the breeding season, the partner provides parental care and is therefore beneficial for current reproduction. After the breeding season, a partner may not be essential for future reproduction since a new partner could likely be found before the next breeding attempt. Indeed, male and female breeding vacancies were often filled quickly and both males and females have been observed to desert their current partner in favour of a vacancy in a neighbouring better-quality territory (Baeyens 1981a). Still, many pairs stay together across multiple years and pairs defend their territory together against predators, conspecific and heterospecific competitors (Chapters 2 and 3, Baeyens, 1981b, 1981a; Birkhead, 1991). Considering that the coordination of calls in pairs already appears to alter how an intruder is perceived (Chapter 4), the strength of the defence would likely be weakened if the partner dies. Furthermore, longer pair bond duration was associated with higher reproductive success in other bird species (Fowler 1995; van de Pol *et al.* 2006; Sánchez-Macouzet *et al.* 2014; Bebbington & Groothuis 2023). Magpies may likewise gain future reproductive benefits from warning their partner also outside of the breeding season. Such protection of the partner has also been suggested for other bird species (Witkin & Ficken 1979; Hogstad 1995; Zaccaroni *et al.* 2013; Stephan & Zuberbühler 2016). It would be interesting to explore to what extent joint territorial defence, year-round territoriality, and benefits of long-term pair bonds correlate with partner protection through alarm calling across species.

Second, warning signals also occurred in over half of the trials with flocks (Chapter 3). Since magpies are reported to form pair bonds already in flocks (Baeyens 1981a), future reproductive benefits may explain alarm calling also in flocks. However, magpies may also call to warn kin for indirect benefits (Hamilton 1964; Maynard Smith 1965). Juvenile magpies have been reported to stay with their siblings and in flocks close to their natal territory at least initially after they dispersed (Baeyens 1981a). Thus, it is likely that siblings were tested together in at least some trials on flocks. Magpies may therefore emit warning signals to gain indirect fitness benefits from warning kin. If magpies may further alarm call to warn and protect group members is unclear. We do not know what group benefits magpies gain from being in a flock, and if these would be worth to warn flock members. Individuals may, for example, join flocks to gain more protection from predators or to gain access to food sources on defended territories (Baeyens 1981a; Clutton-Brock 2002). However, my impression of flocks in our experiments was that there was little cohesion between flock members. The way that we differentiated flocks from families or multiple pairs for our experiments was the mostly independent movement of most individuals in flocks (Chapter 3). That magpies leave the trial area in different directions or fly-in independently, suggests that those flocks are not stable groups in which protecting group members could be beneficial (as, for example, in fairy-wrens (*Malurus coronatus*); (Teunissen *et al.* 2020). Only few studies attempted to assess if group benefits rather than kin-selected or sexually selected benefits likely explain alarm calling in groups, with varying success and conclusions (Cheney & Seyfarth 1985; Smith 1986; Randall *et al.* 2000; Teunissen *et al.* 2020). Eurasian magpies are a promising study system to explore what type of benefits likely drive the evolution of alarm calling in flocks because of the variable group structures and no reproduction occurring in those groups. If flock members were individually identifiable and their relatedness and social bonds known, one could test, for example, if warning signals more likely occur in presence of kin or a future mate. More research will thus be needed to unmask the different benefits that may explain warning signals in groups.

(6.1.4) How to infer meaning from general alarm calls

Since magpie chatter calls are produced across such a broad range of contexts, they categorise as “general alarm calls” (Chapter 1.2; Dezecache & Berthet 2018). Such signals are produced by many species, and their broad usage raises new questions.

First, one may think that these alarm calls can only contain unspecific information, such as a general “alert” (Dezecache & Berthet 2018; Fichtel 2020). Such an “alert” signal would merely elicit receivers to become vigilant. For example, studies on Australian magpies measured changes in the beak angle to ascertain that individuals look up and stay alert for longer on hearing an aerial predator call (Pell *et al.* 2018). In marmots, a subtle “look around” could be differentiated from no response and obvious “rear up and look” in measuring response strengths to different stimuli (Blumstein 1999a). In our studies, magpies were often hidden in vegetation, and it was impossible to reliably focus on the same individual over multiple minutes if they were moving around, lacking individual identifiers. It therefore remains to be tested if a chatter call without additional cues merely elicits an increase in vigilance in magpies.

However, the function of general alarm calls and the behavioural responses to the call-eliciting stimuli can greatly differ (Chapters 6.1 to 6.3). When emitting the chatters, magpies sometimes closely approached a predator or conspecific, other times they stayed further away (Chapter 2). Further, even the same type of threat elicited different response behaviours depending on the social environment (Chapter 3). A series of chatters that were recorded during simulated territorial intrusions and played back at magpie pairs actually elicited receivers to approach and emit many chatter calls in response, even though no threat could be visually identified (Chapters 4 and 5). How can receivers of such general calls ascertain the meaning of the call?

One possibility is that a chatter call indeed elicits receivers to become alert, look around, and infer meaning from the combined visual and auditory cues. The response of the receivers will then depend not only on the call, but also on contextual cues, i.e. if they see a threat that may have caused the stimulus (Fischer 2013; Scarantino 2013; Stephan & Bugnyar 2013). For example, putty-nosed monkeys were shown to gaze more at the speaker (to gain more information from the caller), the sky or elsewhere (to find the cause of the call) in response to calls if they were coupled with additional contextual cues, i.e. the call of an eagle and a non-dangerous disturbance (falling of a tree) (Arnold & Zuberbühler 2013). When we played back series of chatter calls to magpies (Chapters 4 and 5), we often observed magpies to intensely stare at the shrub in which the speaker was hidden, and in some cases, they even moved around the shrub. They may have initially scanned the area to see if a predator is causing a magpie to chatter. But, as no predator could be seen, they appeared to direct their attention at the “caller”. After all, chattering from an unknown individual is a clear sign of an intruder in the territory, which usually requires a territorial defence response directed at the caller (Bradbury & Vehrencamp 2011). Visual cues and contextual information in which calling occurs can thus aid in understanding the likely meaning of a general call (Rendall *et al.* 1999; Wheeler & Fischer 2012; Wheeler & Hammerschmidt 2013), though more experiments are needed to test these hypotheses.

Another possibility is that acoustic variation of call type encodes information about the meaning of the call. Indeed, chatter calls emitted in aerial predator trials were, on average, shorter, faster, and had a lower pitch than average chatter calls in ground predator and aerial predator trials (Chapter 2). The rate of calls, graded variation in call characteristics (e.g. the frequency), or the proportion of specific note types over others in call sequences, have been found to indicate different predator types in various species (Blumstein 1999b; Suzuki 2016; Tegtman & Magrath 2020). In our study (Chapter 2), the call variations overlapped between contexts, so that a shorter, faster, and lower-frequency chatter call unlikely signals a specific type of threat. However, short chatter variants may still function primarily as alert signals to draw the attention of magpies in the vicinity to the presence of a dangerous threat. Longer calls, on the other hand, may indicate that the caller is trying to repel a predator or conspecific intruder. We observed that mostly pairs and families mob predators (Chapter 3). Hence, a series of calls from an intruder (as simulated in Chapters 4 and 5) is unlikely to be perceived as a predator mobbing signal. I would thus predict a territorial defence response from the territory holders to a series of chatters. Indeed, we observed magpies to approach the speaker and emit chatter calls when we played back series of chatters (Chapters 4 and 5), which resembles the response to a conspecific intruder, but it is also similar to mobbing a predator (Chapter 2). We need further experiments to explore if playback of a single short chatter call, for example to a flock, mainly increases vigilance and is thus perceived primarily as an alert signal. By contrast, playing back mobbing calls from the partner rather than calls from an unknown individual may elicit searches for a ground predator. By exploring call variations and in which contexts they are emitted, we may be able to infer more specific meaning to general alarm calls.

If any calls from an unknown individual inside the territory elicit a territorial response, this may also have implications for other highly territorial study systems. We have shown that the behaviours during territorial intrusions and predator mobbing can be very similar (Chapter 2), and mobbing calls may partly function to recruit more mobbers (Curio 1978; Carlson & Griesser 2022). That makes it difficult to disentangle if receivers then call and approach because they perceive the call as indication for a predator or to repel the intruder. Furthermore, also the response to warning signals may be affected by territoriality. After all, the standard way to test if calls are referential is to play them back to an individual or group. If the calls trigger the same escape response as the threat that elicits those calls, then they are clearly perceived as a warning signal (Macedonia & Evans 1993; Manser 2009; Gill & Bierema 2013; Smith 2017). If, however, the call does not elicit the expected response, one reason could be that the receiver does not adhere to the warning call from a stranger in the territory. For example, in vervet monkeys (*Chlorocebus pygerythrus*), alarm barks were only interpreted as warning signal and elicited escape responses in some individuals, whereas they mostly elicited receivers to gaze towards the speaker, especially if the caller was unfamiliar, and potentially even to approach it (Price *et al.* 2014). Further studies found that also the reliability of a caller may alter the responsiveness of conspecifics (Cheney & Seyfarth 1988; Blumstein 2007). However, if the cost of not responding to an alarm call is greater than the cost of escaping without need, then even deceitful alarm calling, for example to steal food from the receivers, can be evolutionary stable (Ridley & Raihani 2007; Wheeler 2009; Flower 2010; Flower *et al.* 2014). There are, hence, more factors that can influence how individuals respond to a call than its inferred meaning.

(6.1.5) Potential for high context-specificity

Even though the chatter call in magpies is used across a variety of context, other calls could still be highly context-specific and perception-specific. I was limited in the experiments I could conduct and vocalisations and contexts I could explore in my thesis. We tested the response to two different predator types in our experiments, with models of a fox (*Vulpes vulpes*) or polecat (*Mustela putorius*) as ground predator and a sparrowhawk (*Accipiter nisus*) as aerial predator. However, I was not able to test responses to flying raptors. A flying raptor that suddenly appears in the sky likely poses a much greater threat than one perched in a tree or on a roof (Cresswell 1994a; Petracca & Caine 2013). Correspondingly, some species were shown to flee and emit a warning call for a flying raptor, while they approached the predator model with mobbing calls when the predator was perched or the model landed on the ground (Griesser 2008; Cunningham & Magrath 2017). It remains to be tested if magpies would similarly elicit a different call and behaviour in such a situation. However, North American black-billed magpies (*Pica hudsonia*), close relatives of the Eurasian magpies, were observed to use a short chatter variant mostly when warning about flying raptors and a longer chatter mostly when mobbing predators (Stone & Trost 1991). It thus seems more likely that also Eurasian magpies warn about highly dangerous threats by varying chatter characteristics than emitting different calls.

Still, magpies produce a variety of calls beyond chatters. Certain vocalisations are often accompanied by visual displays, such as tail-flicking, wing-flirting, or begging postures. My observations suggest that short calls, potentially accompanied by visual displays, were used in social communication with the partner. Further, various harsh calls (often in synchrony with tail-flicks, head-bows or wing-flirts) were also produced during simulated territorial intrusions or during fights between magpie pairs. Many of these calls vary greatly in structure so that we could not reliably classify them, and I focussed on the most common, loud, and clearly identifiable chatter call in my thesis. Still, in some species, even those lacking predator-specific alarm calls, food calls were classified as highly context- and perception-specific (Clay *et al.* 2012; Smith 2017). In fowl (*Gallus gallus*), males emit specific calls when they find food, especially when females are in the vicinity, and these calls elicited searching for food in hens (Evans & Marler 1994; Evans & Evans 1999, 2006). Also chimpanzees and bonobos are reported to produce specific food calls that affect foraging behaviour in receivers (Slocombe & Zuberbühler 2005; Clay & Zuberbühler 2011). Similarly, magpies may also produce context- and perception-specific calls in feeding contexts. While I focussed on the function and specificity of one alarm call in magpies in my thesis, a variety of other calls remain to be explored.

(6.2) The meaning of call variation in an alarm and territorial defence signal

Both general and highly context-specific calls can entail graded variation (Manser *et al.* 2002; Suzuki 2016). In case of the chatter call in Eurasian magpies, we have seen that it can vary in the number of syllables it contains, the rate at which these syllables are emitted, the peak frequency, but also how many calls are emitted at what rate or even the timing in relation to another individual's calls (Chapters 2, 4 and 5). Why may calls vary and what does this variation mean in different contexts?

(6.2.1) Call variation in predatory contexts

In predatory contexts, graded variation in calls often relates to the urgency of a threat. When a predator is closer, many species emit calls at a higher rate, of longer or shorter duration, or at higher or lower pitch (Blumstein 1999b; Wilson & Evans 2012; Suzuki 2016). In some species, such variation also relates to the danger level of a threat, with more or longer calls emitted towards a more dangerous predator (Blumstein 1999b; Yorzinski & Vehrencamp 2009). Such variation in calling could merely be an expression of the arousal or fear experienced by the caller (Marler *et al.* 1992; Owren & Rendall 2001; Fichtel & Hammerschmidt 2002; Wheeler & Fischer 2012; Schamberg *et al.* 2018). In humans, certain variations in our voice, such as the intensity and pitch, are related to emotions (Briefer 2012; Giddens *et al.* 2013). But even if such variation is purely an expression of the internal state of the caller, receivers can make use of this information. In case of alarm calls about predators, a higher call rate may indicate an urgent threat (Manser *et al.* 2001).

In Eurasian magpies, we observed that certain call features varied between predator types (Chapter 2). Our results suggest that shorter and fewer calls were produced towards the presumably greater threat, i.e. the aerial predator, whereas we observed a great variation in chatter calls to ground predator models. If call duration and rate is mainly driven by the affective state of the caller, this would mean that the shorter chatter calls are associated with a more aroused or fearful caller (Briefer 2012; Schamberg *et al.* 2018). However, longer call duration and higher call rate is usually associated with a more aroused caller and more urgent threat (Baker & Becker 2002; Manser *et al.* 2002; Yorzinski & Vehrencamp 2009). The reason for this could be that the anti-predator responses and functions of chatters in our aerial and ground predator trials differ (Chapter 6.1): Aerial predators appear to trigger a more cautious response with very few short chatters that likely function as a warning signal, whereas ground predators appear to be mobbed with longer chatters (Chapters 2 and 6.1). When distinguishing between the likely functions of those calls, we find also other species to emit shorter call variants in alarm only or highly dangerous contexts than in mobbing or less dangerous contexts (Stone & Trost 1991; Cherry *et al.* 2001). Indeed, warning signals are supposedly short, whereas mobbing signals can be longer, harsh and conspicuous to deter the predator (Morton 1977; Klump & Shalter 1984). The variation in the magpie chatter call is hence not (only) related to threat urgency but the function of the call.

Still, also in magpies, call duration may be associated with the arousal of a caller urgency of a threat in the mobbing context. Even though we did not systematically test for an effect of the urgency of a threat on magpie chattering, the distance at which we placed models from the magpies varied between trials (Chapters 2 and 3). Magpies approached the model significantly closer and emitted more chatters when the model was placed closer to the magpies. The duration and pitch of single chatter calls, however, was not significantly affected by the distance between the model and closest magpie (Chapter 2). Future studies could explore more systematically if chatter call duration increases with the urgency of a threat, e.g. the distance of the threat to the caller.

(6.2.2) Call variation during territorial defence

In competitive interactions for resources such as territories, variation in vocal signals is a widespread measure to estimate the strength or motivation of a competitor (Bradbury & Vehrencamp 2011). Competitive displays can entail a variety of signals used throughout the interaction, indicating, for example, the strength of the signaller and the levels of escalation, from low threats to physical attacks (Bradbury & Vehrencamp 2011). Signals may also relate to the arousal of the caller, for example, when challenged by a stronger intruder or when the opponent does not retreat (Smith & Smith 1996; Collins 2004; Slocombe & Zuberbühler 2007; Bradbury & Vehrencamp 2011; Schamberg *et al.* 2018). Signals may then similarly vary depending on the arousal during territorial defence and arousal when mobbing a predator with the same call type. How signals encode such information can be greatly species-specific.

In songbirds, a multitude of studies explored how specific song characteristics relate to competitive abilities or aggressive intend, using various methods (Chapter 1.3.). Traits like repertoire size, switching rate between different song types, or matching the challenger's song type have been shown to be positively associated with aggressiveness or strength of signaller and challenger in a variety of studies (Gil & Gahr 2002; Collins 2004). However, the Eurasian magpie song is comparably soft and rarely produced during simulated territorial intrusions. Hence, it unlikely functions as a territorial signal (Birkhead 1991; pers. obs.). Instead, the "chatter" alarm call is frequently produced towards conspecific territorial intruders (Chapter 2). Many common song measures can thus not be applied to a songbird that mostly produces a single call type to repel competitors.

Furthermore, it also matters how a call is perceived that can be both a predator warning and a territorial signal. In predatory contexts, very few short chatter calls are suggested to alert magpies of a threat being present (Chapter 2). To what extent a short chatter may also alert the receiver when emitted by a territorial intruder is unclear. When we played back chatter calls to magpie pairs, the initial response on hearing an alarm call may be driven by the potential warning function in a short chatter call (Chapters 2 and 5). Yet, the playback contained more calls in a minute than observed in a presumably high-danger context. Hence, magpies approached the loudspeaker and chatter-called in a similar way as they did when exposed to the model of a magpie. It thus seems that also series of short chatter calls are perceived as a territorial threat.

In territorial signalling, call duration may relate to the strength or aggressiveness of the caller (Vehrencamp 2000; Collins 2004). We found considerable variation in the duration of chatter calls emitted during territorial intrusions (Chapter 2) and hypothesise that those may relate to the strength, arousal or aggressiveness of the caller (Chapter 5). However, our results were not conclusive. We found that magpies responded faster to playbacks of long chatters than short chatters, yet other measures of response strength did not differ between playback types (Chapter 5). Nevertheless, magpies differed in the duration of their first chatter between two playback types in another experiment (Chapter 4). Thus, there seems to be some information in the duration of a chatter call. Also in other songbirds, the duration of specific song elements were shown to increase when challenged by an intruder, or to correlate with measures of physical condition of the singer and aggressive intent

(Dabelsteen & Pedersen 1990; Galeotti *et al.* 1997). Duration of vocalisations may thus be one way to signal strength or arousal of the caller during territorial intrusions.

More commonly, the song rate in passerines, call rate of non-passerine birds or generally the rate of threatening displays are associated with competitive contexts, physical attributes or physical aggression (Galeotti 1998; Collins 2004; Garamszegi 2005; Bradbury & Vehrencamp 2011). When we tested for an effect of the rate of calls, magpies did not respond stronger to a high rate than a low rate of short chatters (Chapter 5). However, we found a moderate correlation between the number of chatter calls emitted in response to playbacks and the closest approach distance to the loudspeaker (Chapter 4). It may be that already the low rate of chatters were perceived as a strong opponent or that the high rate of short calls is unnatural and mainly longer chatters are emitted at high rate (Chapter 5). In addition, a receiver may feel inferior to the simulated stimulus and therefore not approach closely or not use highly aggressive signals in response (Collins 2004; de Kort *et al.* 2009; Moseley *et al.* 2013). Consequently, the response to a simulated intruder depends not only on the signal but also the receiver, resulting in varied responses to the same stimulus. This in in-line with our observations (Chapters 4 and 5). Hence, experiments with more playback types varying in chatter duration and rate would help to gain a better understanding of how call duration and rate are perceived by magpies and to which attributes or states of the caller they may relate.

I focussed on understanding variation in the chatter call in my thesis and used common measures, closest approach distance and amount of signalling, to measure the response strength to different playback types. Yet, many competitive interactions entail a variety of different displays, potentially at different stages of an interaction (Bradbury & Vehrencamp 2011). Some songbirds switch from loud, long-distance territorial signals (i.e. territorial song) to other short, soft or visual signals when challenged and during escalating stages of attacks (Bradbury & Vehrencamp 2011; Araya-Ajoy & Dingemanse 2014; Moran *et al.* 2018). Magpies also produce visual signals and other short calls during territorial intrusions (pers. obs.). There is thus much more to be learned about the functions of different signals during territorial displays and defences against intruders, in which alarm calls can play a prominent role.

(6.2.3) Pair coordination during territorial defence

I show that even birds of the Northern hemisphere defend their territory together and may coordinate their signals. We do not know yet if magpies intentionally overlap their chatters or call independently from each other. The strong correlation between number of overlapping chatters and amount of chattering may indicate the latter (Chapter 4). Since magpies in my studies were not ringed and often changed positions or were hidden in vegetation during the trial, we could not assign chatter calls reliably to different individuals over the whole trial, which makes common measures to identify if pairs purposely overlap another's calls impossible(Masco *et al.* 2016). Still, I observed on multiple occasions that a magpie overlapped most of the partner's chatters in a series of chatters, leading me to believe that this could be a coordinated behaviour. Even if magpie pairs call independently from each other, we show that the total signal is perceived differently and elicits a different response when calls overlap or alternate (Chapter 4). If they overlap their syllables precisely, they may not only signal greater coordination (as proposed for duets; Brumm & Slater 2007; Hall & Magrath 2007), but also increase the intensity of the calls (de Araújo *et al.* 2011; Rehberg-Besler *et al.* 2016). Louder songs or song elements were indeed shown to elicit a stronger response in territory owners (Lampe *et al.* 2010; Brumm & Ritschard 2011; Ritschard *et al.* 2012). On the other hand, if members of the pair alternate their calls, they increase the total signalling time, which could also indicate a stronger signal (Vehrencamp 2000; Collins 2004). We found that the initial vocal response indicates overlapping chatters to be perceived as a stronger signal, whereas the non-significant tendency to approach the loudspeaker more closely in alternating chatter playback trials points in the opposite direction. Thus, the perceived signal is altered depending on pairs overlapping or alternating each other's calls.

In duetting birds, the main function of such duets is assumed to be the joined resource defence (Todt & Naguib 2000; Hall 2004; Logue 2005; Dahlin & Benedict 2014). Males and females coordinate their vocalisations in such duets to defend the shared resources (Hall 2004, 2009). Indeed, experimental studies have shown that playback of a pair rather than of a male or female alone is perceived as a stronger opponent and elicits a stronger territorial or aggressive response in receivers (Hall 2000; Molles & Waas 2006; Koloff & Mennill 2013). If this pair not only signals together but also coordinates vocalisations in precise overlaps or alternating vocalisations in a duet, it may signal greater pair coordination and hence a stronger pair (Brumm & Slater 2007). However, playbacks of duets with greater temporal precision in comparison to uncoordinated duets, or playbacks of duets versus male plus female solo songs, did not always trigger a stronger territorial or aggressive response in receivers (Hall & Magrath 2007; Kovach *et al.* 2014; Diniz *et al.* 2021). Also the types of signals (e.g. approach the speaker or duetting in response) varied in strength even to the same signal (Diniz *et al.* 2021), similarly to measured responses to our alternating versus overlapping playbacks (Chapter 4). This shows that evidence for signalling pair strength through coordinated vocalisations within a pair is inconclusive both within and across duetting species.

Another proposed function of duetting is to communicate within the pair (Hall 2004, 2009; Dahlin & Benedict 2014). Such communication, and also female-specific calls, may function to strengthen the pair bond, prevent the partner from being usurped, and to signal commitment between duet partners (Brumm & Slater 2007; Dahlin & Benedict 2014; Amy *et al.* 2018). Similarly, magpie males and females may advertise their own participation in the territorial defence to the partner by emitting chatter calls. The mechanisms how such commitment may be encoded could be diverse. I suggest that the total signalling time, the latency to respond to the partner's calls, or the accuracy with which the partner matches the timing and rhythm of the partner's calls may be perceived as greater commitment. Yet, testing what may be perceived as greater commitment will be difficult, since classical mate-preference tests are usually conducted before pairs are formed and they defend resources together, (Catchpole & Slater 2008). Still, I hypothesise that females signalling with their partner during territorial defence, be it coordinated or independent in timing from the partner's calls, may function not only to defend the territory against outsiders but also to signal commitment to the partner.

Magpies can also be observed to seemingly reply to the partner's calls in other context, where vocalisations are often also combined with visual displays, such as bowing the head and wing-flirts (pers. obs.; Baeyens 1979). I frequently observe magpies to emit soft "dark" calls as they are foraging, when their partner may be some 20 meters away, or one magpie emits very short, higher-frequency calls before taking flight, when the partner usually follows within half a minute. During one territorial intrusion trial, I captured how one magpie always emitted a specific call and the partner responded immediately with another call accompanied by a wing-flirt over a few minutes. These personal observations indicate that magpies appear to communicate much within the pair and coordinate their behaviours, both within and outside territorial contexts. Little is known about the song of magpies, yet some reports indicate that magpies might duet in their babble-song, when they emit a series of alternating calls in which the female answers the male (Baeyens 1979). To what extent magpies may qualify as duetters remains to be determined, but my observations indicate that may express some sort of coordinated vocalisations.

Like many duetting species (Hall 2009; Logue & Hall 2014; Tobias *et al.* 2016), magpie pairs are year-round territorial and often stay together across multiple years (Baeyens 1981a; Birkhead 1991). Both sexes engage in territorial displays (such as sitting and bowing in the treetops) and call at and approach territorial intruders together (Baeyens 1979, 1981b, a; Chapter 2). This alone already highlights the active role females and not only males can take in resource defence (Illes & Yunes-Jimenez 2008; Amy *et al.* 2018; Cooper *et al.* 2023). Recently, though, analyses revealed that male and female song is the ancestral state (Riebel *et al.* 2005; Odom *et al.* 2014), and that song can fulfil wider social functions and is not always a territorial signal (Loning *et al.* 2023). By taking a more inclusive view of coordinated pair behaviours, we may find examples of duet-like behaviours in a greater variety of species and consequently broaden our understanding why such behaviours evolved.

(6.3) What characterises complex vocal communication?

Characterising the complexity of vocal communication is essential when comparing species, for example to understand the evolution of communication systems (Fischer *et al.* 2017). It is still unsolved how and why humans evolved seemingly higher cognitive abilities and complex languages to communicate (Fedurek & Slocombe 2011; Schamberg *et al.* 2018). That is why researchers attempt to find similarities in communication systems between non-human animal species and humans and phylogenetic correlations with other traits or ecological factors (Marler *et al.* 1992; Fedurek & Slocombe 2011; Suzuki 2016; Schamberg *et al.* 2018). The social complexity hypothesis is among the most widespread hypotheses to explain the evolution of complex communication systems and high abilities in social cognition (Freeberg *et al.* 2012; Fischer *et al.* 2017; Schamberg *et al.* 2018). However, defining and reliably quantifying complexity is challenging (Fischer *et al.* 2017).

Historically, much research focussed on quantifying the vocal repertoire. Song complexity has been compared between passerine species by counting the number of distinct song elements that are produced, how many different elements are produced in one song type, and how many different song types are produced (Catchpole & Slater 2008). Also, non-song vocalisations of a species have mostly been classified based on distinctiveness, and linked to contexts in which they are commonly produced (Bradbury & Vehrencamp 2011; Freeberg *et al.* 2012; Kershenbaum *et al.* 2015). However, making distinctions between call types can be very challenging if there is, for example, a lot of variation in the duration, frequency, or (temporal) features of a call, so that various vocalisations may be on a continuum between different call types (Fischer 2013). Researching primate vocalisations can be a challenge for such reasons, and I encountered similar difficulties when I attempted to classify non-chatter vocalisations in magpies. Forcing calls into distinct categories and counting those categories only would neglect the complexity of variation within categories (Fischer *et al.* 2017).

When the idea of referential calling arose, researchers may have deemed a communication system as more complex if a species uses multiple highly context-specificity call types for specific threat types only (Macedonia & Evans 1993; Freeberg *et al.* 2012; Manser 2013). A "general" alarm call used across a range of contexts and with seemingly low perception-specificity (e.g. eliciting only a "general alert" and no specific behavioural responses) then appears less complex (Marler *et al.* 1992; Dezecache & Berthet 2018). However, researchers pointed out that the cognitive demand of associating a specific call type with a certain threat is probably rather low (Wheeler & Fischer 2012). In contrast to that, it was suggested that integrating information from various cues to understand the meaning indicates higher complexity and greater cognitive abilities (Wheeler & Fischer 2012; Fichtel 2020). To understand the meaning of the signal, more than the acoustic modality and contextual cues may need to be investigated (Seyfarth *et al.* 2010; Suzuki 2016). While many words refer to a specific object in human languages, words often also have different meanings depending on the context they are spoken in. Likewise, the chatter call in magpies appears to take different meanings depending on the context it is used in. It contains usually only one syllable type that does not appear to have a complex tonal structure. Still, it is used across various contexts in which the function appears to differ (Chapter 6.1), making it even more intriguing to understand how individuals can understand the specific meaning of a call. I hypothesise that magpies likely assess a variety of contextual (e.g. visual cues, social context), and call parameters to interpret the meaning of a chatter call and respond accordingly. My thesis thus highlights that there can be great complexity even in a single call type.

Historically, non-human animal calls were interpreted as mere expressions of the physiology or internal state of the caller, in particular contrast to human speech (Smith 1977; Darwin 1872/1998). However, previous studies, as well as my thesis, have shown that many vocalisations, also general calls that may be tightly linked to the arousal of an individual, are not always emitted when facing a threat but only in specific social contexts to inform or protect certain individuals (Marler & Evans 1996; Fichtel & Manser 2010; Schamberg *et al.* 2018). To some extent, the arousal in face of a predator may be increased if vulnerable offspring are threatened, or aggressiveness may be increased during the breeding season (Cully & Ligon 1986; Wingfield *et al.* 1987; Schamberg *et al.* 2018). In primates, however, studies have shown that callers may aim their calls at specific individuals so that they only stop alarm calling until their partner or all group members responded to the call and thereby signalled that they are aware of the threat (Wich & de Vries 2005; Stephan & Zuberbühler 2016). In chimpanzees, the specific social context in which an alarm call is emitted has been used to suggest that they possess some "theory of mind": They show that alarm calls are emitted mainly when group members are unaware of the threat and not if they know the threat is there (Crockford *et al.* 2012). That means that chimpanzees appear to understand what other group members know and act accordingly. Even the usage of a general alarm call can therefore indicate specialised functions of calls and hint at cognitive abilities (Cheney & Seyfarth 2018).

Moreover, information about the physiology an internal state of the caller can be complex, of great biological importance, and is ultimately not dissimilar from humans (Bradbury & Vehrencamp 2011; Filippi *et al.* 2017). It is essential for individuals to be able to recognise their own species, the sex of an individual (e.g., revealing it as potential mate or competitor), or the state (e.g. level of arousal) of an individual (Becker 1982; Bradbury & Vehrencamp 2011). Also humans can identify the sex and approximate the age of another human from the voice alone, and we are able to recognise a vast number of individuals by their voice (Giddens *et al.* 2013).

For both humans and non-human animals, arousal has been associated with vocal characteristics, such as the pitch, the intensity of vocalisations, or rate at which they are emitted (Briefer 2012; Giddens *et al.* 2013; Filippi *et al.* 2017). Even though such variation may be caused mainly by the internal state of the individual, it can still contain useful information for other individuals (Bradbury & Vehrencamp 2011). For instance, receivers may learn about the urgency of an external threat (Marler *et al.* 1992; Suzuki 2016). Further, especially in group-living species, assessing the state of conspecifics can be essential to appease or avoid a group member that is aggressive (Bradbury & Vehrencamp 2011; Schamberg *et al.* 2018). Also in competitive interactions, assessing the state of the opponent is essential to signal back, asserting strength, arousal, or motivation to escalate a fight and thereby prevent losing in competition, or, on the other hand, signal submission and avoid the cost of attack (Bradbury & Vehrencamp 2011). Overall, in many systems including humans, gaining more detailed information on the state of the caller from graded variation in vocalisations can be essential.

In Eurasian magpies, I showed that it can be difficult to understand if or what kind of information may be encoded in variation of signals (Chapters 1, 4,5 and 6.2). Yet, only because our results on the meaning of chatter variations were inconclusive, it does not necessarily mean that a signal does not carry information. Even when learning foreign languages, there are many subtleties, expressions and 'between-the lines' meanings that can only be understood through knowing the language and culture well and getting explanation. To understand non-human animals, we can only measure acoustic characteristics in a variety of contexts and individuals, or perform experiments manipulating contexts or hormone levels to correlate vocal characteristics with specific external referents, behaviours, individual characteristics or states. In this thesis, I conducted a first set of experiments that revealed the complexity of the graded variation in chatter calls and enabled me to speculate about their likely functions.

Taken together, vocal communication can be complex in many ways, and the perspectives and methods to define it have changed over the last century. Some of the standard observations and experiments (e.g. observing call production in different contexts or observing responses to different playbacks) are still an essential step to gain understanding of what a signal could mean. Yet, the system may be too complex to fully understand it with a small set of experiments only. It is further essential to explore which evolutionary drivers, ecological aspects and species-specific traits and constraints shape vocal communication. After all, the vocalisations we observe now are a product of adaptations to the ecological and social environment of a species. This also means that by comparing how vocalisations (i.e. distinct types and 'graded' variations) are used across contexts, may carry what kind of information, or aim to elicit what kind of responses in receivers, elucidates what drives the evolution of such signals.

I focussed on a single call type in a single species in my thesis to understand the function and complexity of this signal. Still, my research highlights that there is complexity in how this call type can vary in its structure and the rate at which it is used, the information it may carry, and the function it may fulfil in different threatening and social contexts. It thus contributes to how we perceive and define complexity and further entails suggestions for various fields.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Summary

Acoustic communication is widespread across the animal kingdom and is used across a variety of contexts. Especially vocalisations produced in predatory contexts, i.e. alarm calls, have been studied throughout various scientific disciplines. They are intriguing from a functional and evolutionary perspective, for example to understand what benefits the caller would gain from warning conspecifics at a potential cost to itself. Moreover, researchers have been exploring what kind of information may be conveyed by alarm calls. May alarm calls refer to external objects or events similarly as words in human languages? Or are they an expression of the affective state of the caller (e.g., arousal or fear)? Does the caller emit an alarm call to trigger a specific response in the receiver, e.g., to flee or approach and help? Beyond that, it is unclear why only some alarm calls are highly context-specific, i.e. they are produced only in response to specific predator types, and elicit a receivers to respond as if they encountered the predator themselves. In contrast, many other calls are produced across a variety of contexts. Moreover, many of such "general alarm calls" are produced not only in predatory contexts but also in interactions with conspecifics. What kind of information may then be conveyed through general alarm calls and their variation in different contexts, and which variations are meaningful to receivers?

In my thesis, I tested a range of hypotheses relating to these questions, using the Eurasian magpies (*Pica pica*) as model system. I tested how magpies use, vary, and perceive their characteristic "chatter" call, which is a loud rattle of short, harsh, broadband syllables that are repeated at a high rate. While alarm calls have been investigated in many bird species, the vocalisations of Eurasian magpies provide a particularly intriguing system to study vocal communication. Magpies likely have large vocal flexibility, as they can learn to mimic sounds, and have high cognitive abilities. Thus, magpies would likely have the potential for highly complex acoustic communication. Nevertheless, they appear to use the chatter call over a wide range of predator-induced as well as territorial contexts. Therefore, I tested the function and specificity of the chatter call of magpies and assessed whether relevant context-specific information is encoded in chatter variation.

In **Chapter 1,** I introduce the topic and research aims**.** In Chapters 2 and 3 (see below), I tested experimentally how magpies respond and use their chatter calls in threatening contexts induced by predator or conspecific taxidermic models, while in Chapters 4 and 5 (see below), I tested experimentally how magpies respond to variation in these chatter calls.

In **Chapter 2**, I tested experimentally the context-specificity of the chatter call and chatter variations. For this, we exposed magpie pairs to taxidermic models of (i) two different predator types: a fox representing a predator hunting on the ground, and a perched

sparrowhawk representing an agile aerial predator that can also pursue birds in flight. In addition, we measured approach, threatening and chatter responses to (ii) taxidermic models of Eurasian magpies, representing a territorial intruder, and (iii) pigeons as a control. We found that magpies responded strongest to conspecific intruders, with high rates of chatters, close approaches, occasional threatening displays (e.g. dives over the model), and attacks (i.e. hitting or pecking the model). In trials with ground predators, magpies also emitted equally high chatter rates, and sometimes closely approached and threatened the model. The response to aerial predators, however, was very similar to control trials with no close approaches and few chatters emitted in only some trials. However, those chatters were, on average, shorter (in number and duration of syllables) and of lower pitch than chatters emitted to conspecific intruders and ground predators, and also differed in syllable duration and pitch from chatters in control trials. Thus, it appears that magpies mob ground predators in a similar way with similar chatters as they respond to conspecific intruders (though with fewer risky behaviours), whereas they may warn vocally about a dangerous threat (aerial predators) with a short chatter variant.

In **Chapter 3**, I assessed the ultimate function of alarm calls by experimentally testing in which social environments magpies intensely chattered and approached models of ground and aerial predators. We found that mostly pairs and families emitted high rates of chatters and closely approached the ground predator. Further, lower rates of alarm calls were produced in over half of the trials in which magpie pairs and families were exposed to an aerial predator, and also in more than half of the trials in which flocks of magpies faced an aerial or ground predator. Single magpies, however, did not signal at any of the predators or called for help as they chatter called in only 4 of 25 trials. It thus appears that magpies warn conspecifics for various social benefits (i.e., securing reproduction, and potentially kin-selected benefits, or group benefits), whereas they appear to mob the predator with high call rates and close approaches mainly to warn or protect their partner and offspring.

In **Chapter 4**, we tested experimentally how chattering is perceived by conspecifics. Previously, we observed magpie males and females to defend their territory against conspecifics and ground predators together, both emitting high rates of chatter calls and often overlapping each other’s calls (Chapter 1). We therefore determined in a two-loudspeaker playback experiment if magpie pairs perceive playbacks of chatter call series inside their territory differently if the playback of one loudspeaker overlapped or alternated (i.e. followed) the chatters of the other loudspeaker. Indeed, magpies responded with a longer first chatter to playback of overlapping chatters. Still, magpies had a non-significant tendency to approach the loudspeakers more closely during playbacks of alternating chatter calls. This indicates that magpies perceived chatter calls differently depending on how a simulated pair coordinates the calls.

In **Chapter 5**, we tested experimentally how variation in the duration and rate of chatter playbacks is perceived by magpies. We found that magpies responded with a first chatter sooner if chatter playback consisted of longer chatters. However, neither the total number of chatters, nor the duration of the first chatter or the approach distance differed according to variation in chatter duration or rate. Magpies thus appear to perceive longer chatters from inside their territory as a more urgent intruder that they need to respond to faster. Likewise, they may be more hesitant in their initial response on hearing short chatters since these could convey a warning in predatory contexts (Chapter 2). This result demonstrates that magpies can perceive variation in the duration of general alarm calls and respond to such variation accordingly.

To conclude, I found that the use and responses to variation of chatter calls is highly complex. Even though chatter calls appear to be produced “generally” in a variety of contexts, their usage and function seem to differ between contexts. Longer chatters and higher rates of chatters appear to mainly function in deterring a predator or signalling at a competitor, whereas a short chatter alone is potentially perceived as a warning signal and may elicit a hesitant response initially, even when emitted by an intruder. Furthermore, magpies potentially integrate information from calls with additional cues for predator presence or with information from the social context in which a call occurs (e.g. perceiving a call from a family member or from an intruder). Even though I can, based on my results, only hypothesise what exactly chatter calls mean or how they encode information, I show that even the production and usage of a single call can be highly complex. This makes the magpie alarm call a fascinating target also for future research. Further exploring the usage of “general” alarm calls, like the chatter call of magpies, will help us to gain a better understanding of why calls are produced and how receivers can infer the meaning of a call. There is great potential for complex meaning even in a general alarm call, for which we must look at how call usage and structure vary among contexts and how call variants are perceived in different contexts.


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(left: "STARE_P1-1" painted by Arnout Lindeman)

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Personal information

- About the Author
- List of Scientific Publications and Presentations
- WIAS Training & Supervision Plan (TSP)

About the author

Miriam Kuspiel was born on 12th July, 1994, in Eschweiler, Germany. In 2010/2011, she went to Brazil on school exchange for one year. She graduated from high school (Städtisches Gymnasium Eschweiler) and obtained her "Abitur" diploma (A-level equivalent) in 2013. Afterwards, she moved to Bielefeld, Germany, to do a Bachelor of Science in Biology (profile behavioural and neural biology, graduated 2016) and consecutively a Master of Science in "Behaviour: from Neural Mechanisms to Evolution" at Bielefeld University (graduated 2019). She completed part of her bachelor courses at the University of Essex in England, within the ERASMUS exchange programme. Miriam conducted research projects in various fields within the evolutionary biology and behavioural ecology departments during both her bachelor and master studies. She conducted her bachelor thesis on the genetic variability of the immune gene Slc11a1 in three pinniped species under supervision of Joseph Hoffman and Emily Humble. For her master thesis, she studied aggressiveness and its relation to territory quality in blue tits (*Cyanistes caeruleus*), under supervision of Peter Korsten and Stephen Salazar, in the Vosbergen study population near Groningen, NL. For another master research project, she joined Meike Zemihn from Leiden University, NL, to study the calls of common marmosets (*Callithrix jacchus*) in the wild in Brazil.

With her prior experience in studying birds and intrigued by the social system of marmosets, Miriam decided to study cooperatively breeding birds with Sjouke A. Kingma at Wageningen University, NL. In 2019, she started her PhD on the future benefits of cooperative breeding in white-crested helmetshrikes (*Prionops plumatus*), under supervision of Sjouke A. Kingma and Marc Naguib. Due to the Covid pandemic, however, extensive fieldwork in Eswatini became impossible for two seasons. She therefore changed her PhD topic to work with birds in the Netherlands and investigated vocalisations and anti-predator behaviours in Eurasian magpies (*Pica pica*).

Miriam started a three-month WIAS Postdoc Writing Fellowship at Wageningen University in June 2024 to write a grant proposal. She plans to do a postdoc on the geographic and temporal variation of bird song in pied flycatchers (*Ficedula hypoleuca*) with David Wheatcroft at the University of Stockholm.

List of Scientific Publications and Presentations

Journal Publications

Salazar, S.M., García, J.I.C., **Kuspiel, M.**, Fokkema, R.W., Komdeur, J. & Korsten, P. (2021) Male aggressiveness and risk-taking during reproduction are repeatable but not correlated in a wild bird population. *Behavioral Ecology and Sociobiology* **75**, 108, DOI: 10.1007/s00265-021-03044-x.

In Preparation

Kuspiel, M., Lindeman, A., Naguib, M., Kingma, S.A. (in prep) The "chatter" call in Eurasian magpies (*Pica pica*) is an alarm, mobbing, and territorial defence signal.

Kuspiel, M., Bebbington, K.L., Naguib, M., Kingma, S.A. (in prep) Who is listening? The function of anti-predator alarm calling in Eurasian magpies.

Kuspiel, M., Kingma, S.A., Vermeulen, H., Naguib, M. (in prep) Pair-coordinated defence: Overlapping calls alter territorial responses in Eurasian magpies.

Kuspiel, M., Lindeman, A., Naguib, M., Kingma, S.A. (in prep) Duration of an alarm and territorial defence call alters receiver response in a corvid.

Conference Proceedings and Abstracts

Kuspiel, M., Bebbington, K., Kingma, S.A. (2023). Chattering magpies: Social context determines alarm calling to threats. At Behaviour 2023 Bielefeld, Germany. Oral presentation.

Kuspiel, M., Naguib, M., Lindeman, A., Bebbington, K. L., & Kingma, S.A. (2023). Variation and similarities in how Eurasian magpies respond to threats. In *WIAS Annual Conference 2023: Surpassing boundaries: Going one step ahead* (p. 24). Wageningen University & Research. Oral presentation.

Kuspiel, M., Naguib, M., Bebbington, K., & Kingma, S.A. (2022). The social context of alarm calls: Function and specificity of alarm calling in Eurasian magpies. At ISBE Congress 2022. Stockholm, Sweden. Poster presentation.

Kuspiel, M., Naguib, M., Bebbington, K., & Kingma, S.A. (2022). Alarm calls in Eurasian magpies: Variability, context specificity and function. In *European Conference on Behavioural Biology 2022: All of life is social!*. Groningen, Netherlands. Poster presentation.

Kuspiel, M., Bebbington, K. L., Naguib, M., & Kingma, S. A. (2021). Moving out: do the costs of extra-territorial movement explain delayed dispersal in cooperative breeders? In *WIAS Annual Conference 2021: 28 – 29 April 2021 Online COVID-friendly edition: Resilience* (pp. 38-38). Wageningen University & Research. Oral presentation.

Kuspiel, M., Kingma, S., Bebbington, K., & Naguib, M. (2020). Stay and help or go (to help)? - Cooperative breeding in white-crested helmetshrikes. In *WIAS Annual Conference 2020: Frontiers in Animal Sciences* (p. 75). Wageningen University & Research. Poster presentation.

Kuspiel, M., Zemihn, M.K., Zijlstra, T.W., Clarke, E., ten Cate, C. (2019). Whistleblower: Variability of call combinations in a cooperatively breeding primate. At *ASAB summer conference 2019*. Konstanz, Germany. Oral presentation.

Kuspiel, M., Schmoll, T. (2016). Age-dependent fertilisation success in tits – A role for sperm design?. At *11th Annual Meeting of the Ethological Society*. Göttingen, Germany. Poster presentation.

WIAS Training & Supervision Plan (TSP)

Activity	Year
The Basic Package (2.9 ECTS[1])	
WIAS Introduction Day	2020
Scientific Integrity	2020
Ethics and Animal Sciences	2020
Introduction to Personal Effectiveness	2021
Disciplinary Competences (7.2 ECTS[1])	
Writing a research proposal	2020
Generalised Linear Models	2022
Collaborative Version Control with Git and GitLab Workshop	2023
Practical assistance in great tit mist netting, measuring and blood-sampling	2020
Professional Competences (10.5 ECTS[1])	
Brain-friendly Working & Writing	2020
Project & Time Management	2020
Research Data Management	2020
Writing Grant Proposals	2020
Systematic Approaches to Reviewing Literature	2021
Start to Supervise BSc & MSc Thesis Students	2021
WGS PhD Workshop Carousel (online)	2021
WGS PhD Workshop Carousel 2022	2022
Intensive Writing Week	2023
The Final Touch: Writing the General Introduction and Discussion	2023
Last Stretch of the PhD Programme & Writing Propositions for your PhD	2023

Presentation Skills (4 ECTS[1])

"Helping for future aspiration: Cooperative breeding in white helmetshrikes", WIAS Annual Conference, Lunteren, poster	2020
"Moving out: Do the costs of extra-territorial movement explain delayed dispersal in cooperative breeders?", WIAS Annual Conference, online, oral presentation	2021
"Alarm calls in Eurasian magpies: Variability, context specificity and function", ECBB 2022 "All Life is Social" symposium, Groningen, poster	2022
"The social context of alarm calls: Function and specificity of alarm calling in Eurasian magpies", ISBE 2022 Stockholm (18th International Society for Behavioural Ecology Congress), poster	2022
"Chattering magpies: Social context determines alarm calling to threats", Behaviour Conference 2023 Bielefeld, oral presentation	2023

Teaching competences (6 ECTS[1])

Supervising course practicals (Animal Behaviour, Behavioural Ecology)	2020, 2021, 2022
Supervision of 1 MSc internship student	2021
Supervision of 2 MSc major thesis students	2021, 2022
Supervision of 1 BSc internship student	2022
Supervision of 2 BSc thesis students	2022

Education and Training Total: 30.6 ECTS[1]

[1] One ECTS credit equals a study load of approximately 28 hours

“An understanding of the natural world and what’s in it is a source of not only a great curiosity but great fulfillment.”

“Birds are the most popular group in the animal kingdom. We feed them and tame them and think we know them. And yet they inhabit a world which is really rather mysterious.”

David Attenborough

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