# THE SENSORY ECOLOGY OF YELLOW WARBLER REFERENTIAL ALARM CALLS

BY

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DISSERTATION

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# ABSTRACT

Diverse animal lineages communicate using complex vocalizations, which can have structural and functional parallels with human language. One such set of communication signals is the functional reference call, which is a form of symbolic communication that has evolved repeatedly in vertebrates, including several times amongst birds. These calls denote specific objects in the environment, generally predators, and elicit behavioral responses from animals that hear these calls that are specific for the threat being referenced (e.g. hiding under cover from flying predators). Understanding the sensory ecology and developmental mechanisms that allow animals to process and respond to complex vocalizations can inform us about the evolutionary forces that drive the formation of intricate communication systems and, ultimately, language. Yellow warblers (*Setophaga petechia*), a small North American passerine species, produce referential "seet" calls to warn of a different type of danger – obligate brood parasitic brown-headed cowbirds (*Molothrus ater*). Brown-headed cowbirds lay their eggs in the nests of other species, leaving these hosts to care for the parasitic young. Yellow warblers produce seet calls in response to brood parasitic signals (cowbirds, their vocalizations, and conspecific seet calls) to warn nearby individuals of this specific danger. Female warblers that hear the seet call return to sit tightly on their nest to presumably prevent cowbirds from inspecting and parasitizing it. Previous work on seet calls in yellow warblers has shown temporal specificity in production of the calls, particularly during the egg-laying stage when cowbirds are most likely to parasitize nests. The majority of literature on yellow warbler seet calling has focused on the production and behaviors associated with seet calls in response to model presentations. However, the sensory-perceptual abilities of birds to discriminate and respond adaptively to vocalizations signaling different threats (e.g. parasite vs predator) and the ecological contexts that promote these abilities, have hardly been explored. I explored these questions by presenting playbacks of cowbird chatters, seet calls, chip (general alarm) calls, predator calls, and controls, to yellow warblers varying in sex and breeding stage, and measuring behavioral responses including latency to respond, alarm calling, and approach, and nest sitting defenses both at the time of playbacks and the subsequent morning. I also explored whether and how heterospecific eavesdroppers utilize and respond to neighboring yellow warbler seet calls as a defense against cowbirds at their own nests. Through the studies of my dissertation, I found that latency to respond, alarm calling, approach, and nest sitting defenses were affected by acoustic stimuli signaling brood parasitism (cowbird chatters and seet calls). Furthermore, similar to model presentations, yellow warblers used seet calls and chip calls towards acoustic stimuli of brood parasites and predators, respectively, with seet call production being mediated by the temporal risk of brood parasitism. Additionally, I found that red-winged blackbirds (*Agelaius phoeniceus*), a heterospecific species that often nests near yellow warblers, eavesdrop on seet calls of yellow warbler neighbors. Red-winged blackbirds also varied in the same behavioral response measures as yellow warblers towards signals for brood parasitism depending on their current risk of parasitism. Thus, the studies in my dissertation further our understanding of the sensory ecology of yellow warbler referential calling, and the functional contexts that both conspecific warblers and heterospecific eavesdroppers use when responding to anti-parasitic referential alarm calls.

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# TABLE OF CONTENTS

# CHAPTER 1: DO HOSTS OF AVIAN BROOD PARASITES DISCRIMINATE PARASITIC VS. PREDATORY THREATS? A META-ANALYSIS

## 1.1 ABSTRACT

Many animals have evolved fine-tuned enemy recognition (the ability to discriminate between threat types) and respond to threats based on their particular impact on survival and/or fitness. Birds represent an important and tractable behavioral study system to explore hypotheses of enemy recognition in detail: in addition to predation risk to adult and nest survival, up to 17% of avian species also face reproductive threats from brood parasitism, whereby parasites lay their eggs in other species' nests. While nest predation is detrimental to progeny fitness throughout the reproductive cycle, brood parasitism can carry different costs depending on the host's nest stage and whether the host rejects parasitic eggs or chicks. We conducted a literature review and a formal meta-analysis of studies that conducted model presentation experiments to compare aggression levels of hosts toward brood parasites vs. predators, and synthesized up-to-date findings on such avian enemy recognition patterns. We focused on whether hosts are more aggressive toward brood parasites during the high-cost laying and incubation stages compared to the low-cost nestling stage, whereas responses to nest predators were predicted to be consistently strong or even increasing toward latter nesting stages. We also evaluated whether these front-loaded defenses prior to the brood parasite's access to nests are modulated by hosts' foreign egg ejection responses (accepters vs. rejecters), brood parasitic offspring strategy (nestmate-evictors vs. nest-sharers), and host-brood parasite geographic overlap (sympatry vs. allopatry). As predicted, hosts responded more aggressively toward models of brood parasites during the laying and incubation stages compared to the nestling stage. In turn, host aggression toward nest predators increased in intensity level during the nestling stage. We also found support that host type mediates anti-brood parasitic responses, in that accepters were generally more aggressive to brood parasites than rejecters. We did not find evidence that geographic overlap significantly affected anti-parasitic responses, but we did find differences based on the brood parasite's nestling strategies (evictor vs. non-evictor). These findings indicate that avian hosts of brood parasites make adaptive decisions regarding costly nest defensive behaviors to protect their offspring depending on the type and cost of the threat.

## 1.2 INTRODUCTION

Animals experience diverse interspecific interactions, including those with “natural enemies” such as predators and parasites negatively impacting survival and reproduction (Morin, 2009; Pollock et al., 2021). Encounters with predators and parasites both reduce fitness, but fitness costs differ based on the type of threat and when these threats are encountered (Raffel et al., 2008). Thus, it would be adaptive for animals to discriminate between natural enemies and choose the behavioral response that minimizes fitness losses. The ability to discriminate among threats is well-studied in predator-prey contexts, with prey species across taxa capable of discriminating between different types of predators (e.g., Burhans, 2001; Chivers & Mirza, 2001; Dorosheva & Reznikova, 2011; Ferrari et al., 2008; Fuchs et al., 2019; McLean et al., 1996). Indeed, animals select among their own repertoire of antipredator strategies based on predator type (e.g., flying vs. aerial; Seyfarth et al., 1980; Blumstein, 1999a; Rainey et al., 2004a,b) or threat level (e.g., size or distance relative to prey; Helfman, 1989; Courter & Ritchison, 2010; Rauber & Manser, 2017), which in turn improves survival (reviewed in Griffin, 2004).

In addition to the recognition of predators that directly threaten their own survival, animals distinguish among threats specifically related to reproductive success, such as predators targeting vulnerable offspring, including eggs and dependent young. Recognizing threats to reproduction is particularly critical for birds, as nest depredation is the leading cause of reproductive failure for this lineage (Chiavacci et al., 2018; Martin, 1992; Martin, 1995). Many avian species respond aggressively to nest predators to improve reproductive success odds (reviewed in Lima, 2009), recognizing that nest predators pose a high risk to nest survival but little or no risk to the adults' survival (e.g., Oteyza et al., 2021). In turn, up to 17% of bird species must also defend nests against brood parasites, which lay their eggs in the nests of "host" species that must care for the brood parasitic young (Antonson et al., 2020; Davies, 2010). Behavioral responses to both types of nest threats often include aggressive mobbing behaviors to prevent nest depredation or brood parasitism from occurring (referred to as "front-loaded" defenses in the context of brood parasitism; Feeney et al., 2015; Feeney et al., 2012; Kilner & Langmore, 2011; Welbergen & Davies, 2009). However, notable differences in aggression levels toward these threats also demonstrate that avian host species discriminate between nest predators and brood parasites, such that parents adjust their behaviors to match the distinct threats specifically (Burhans, 2001; Enos et al., 2020; Gill & Sealy, 1996; Sealy et al., 1998).

To explore the discrimination of unique threats posed by nest predators and brood parasites, researchers typically frame their experiments around four hypotheses about host enemy recognition. A frequently tested hypothesis about host enemy recognition poses that discrimination between nest predators and brood parasites is specific to the nest stage. Unlike nest predators, which threaten reproductive success when either eggs (referred to as "laying" and "incubation" stages) or nestlings (referred to as the "nestling" stage) are present, brood parasites mostly pose a reproductive threat if they successfully parasitize a nest while the host is actively laying or incubating eggs (Fiorini et al., 2009; Geltsch et al., 2016; Wang et al., 2020). Hence, hosts that discriminate between brood parasites and nest predators should primarily respond to brood parasites with aggression during laying and incubation stages (e.g., Fasanella & Fernández, 2009; Gill & Sealy, 1996; Neudorf & Sealy, 1992). In contrast, nest predators should elicit aggressive responses from hosts at all nest stages because the outcome of nest predation is often the total loss of the reproductive attempt (e.g., Fasanella & Fernández, 2009; Gill & Sealy, 1996; Ruiz et al., 2018). Moreover, aggression intensity toward nest predators often increases as nests progress from incubation to nestling stages, because of the high investment in advanced broods near fledging (Campobello & Sealy, 2010; Montgomerie & Weatherhead, 1988; Regelmann & Curio, 1983).

Host species' aggression intensity toward brood parasites has also been hypothesized to specifically depend on the ability to distinguish brood parasitic eggs from their own (e.g., Davies & Brooke, 1989; Manna et al., 2017; Rothstein, 1986). Several host species physically eject parasitic eggs once recognized (Antonov et al., 2009; Rothstein, 1975; Soler, 2014). These "rejecters" also benefit from front-loaded defenses to prevent brood parasitism from occurring (i.e., aggression toward the brood parasite female to avoid host-egg removal in connection with the brood parasitic egg-laying; Croston & Hauber, 2015). However, non-rejecters, referred to as "accepters," consistently experience higher costs from being parasitized as they do not eject eggs and subsequently allocate resources toward the unrelated obligate brood parasitic young (Hauber, 2003; Kilner et al., 2004; Lichtenstein & Sealy, 1998). The difference in fitness outcomes suggests that accepters should exhibit more front-loaded aggression toward brood parasites than rejecters (Neudorf & Sealy, 1992; Rothstein, 1975; Sealy et al., 1998). In turn, both accepters and rejecters are expected to exhibit similar levels of front-loaded aggression toward nest predators because of the shared and high fitness cost of nest depredation (e.g., Enos et al., 2020).

Though it concerns host aggression rather than discrimination, a third hypothesis poses that host aggression intensity should depend on the competitive strategy utilized by brood parasitic nestlings after hatching. For example, some species of brood parasitic nestlings, including common cuckoos (*Cuculus canorus*) and striped cuckoos (*Tapera naevia*), evict or directly kill all nestmates, typically resulting in total loss of fitness for the host (Davies, 2010; Kilner & Davies, 1999; Mark & Rubenstein, 2013). In contrast, brood parasitic species that do not evict host nestmates, including the brown-headed cowbird (*Molothrus ater*) and the Great Spotted Cuckoo (*Clamator glandarius*), often lead to only partial host-fitness loss as brood parasitic nestlings compete for resources from parents, therefore reducing the survival of some, but typically not all, host nestlings (Hauber, 2003, Soler et al., 2014). As such, hosts may respond differently depending on the species and reproductive strategy of brood parasites, specifically whether they are nest-sharers or nestmate-evictors.

The final hypothesis is that geographic isolation between hosts and brood parasites influences enemy recognition and discrimination among hosts. In areas of geographic overlap or sympatry, hosts should exhibit higher aggression toward brood parasites during the laying and/or incubation stage compared to the nestling stage (as discussed above under the stage-specific enemy recognition hypotheses). In contrast, "naive" or allopatric host populations that have been geographically isolated from brood parasites may not recognize brood parasites as unique threats to reproduction (Briskie et al., 1992; Kuehn et al., 2016). In this case, hosts would respond to brood parasites and nest predators with the same level of aggression and also increase aggression intensity toward both threats during the nestling stage (e.g., Lawson et al., 2020b). Alternatively, host populations that are geographically isolated from brood parasites may not recognize brood parasites as a reproductive threat, and may instead perceive them as non-threatening heterospecific intruders.

Support for these four hypotheses about enemy recognition by hosts largely come from visual, model-presentation experiments (sometimes coupled with acoustic playbacks), where researchers place taxidermic models or effigies of predators, brood parasites, and/or non-threatening control species at nests and then record the host's aggressive responses to model treatments (reviewed in Soler et al., 2017). However, most experiments are not designed to compare host responses across all three model treatments, or across all nesting stages, within a single study (but see Gill & Sealy, 1996). Additionally, most studies only test one or a few host species and only one brood parasite at a time (but see for multi-host species studies: Robertson & Norman, 1976; Moksnes et al., 1991; Sealy et al., 1998). Undoubtedly, host type (accepter vs. rejecter), nest stage (laying, incubation and nestling), threat type (nestmate-evictor vs. nest-sharer, nest predator vs. adult predator) and host exposure to brood parasites (sympatry vs. allopatry) are not mutually exclusive factors influencing host enemy recognition and discrimination. It is thus critical to evaluate all factors at once to better address existing hypotheses about enemy recognition by avian hosts.

We conducted a systematic literature review of studies adopting model presentation experiments to evaluate support for the four hypotheses about host discrimination between predators and brood parasites. We then used a formal, phylogenetically-controlled meta-analysis (Koricheva et al., 2013) to quantitatively test the following predictions for each hypothesis:

1. Host-specific Discrimination: differential aggression in responses of hosts toward brood parasites vs. predators will be greater in species that do not eject parasite eggs (accepters) than in those that do (rejecters).

2. Stage-specific Discrimination: hosts will be more aggressive toward brood parasite models during the laying and incubation stages compared to the nestling stage, whereas responses to predators would either stay the same or increase with the progression of these nest stages.

3. Threat-specific Discrimination: the type of the brood parasite (nestmate evictor vs. nest-sharer) and predator it is compared to (nest predator vs. adult predator) influences host aggression, with nestmate-evictor parasites and adult predators receiving more aggression from hosts.

4. Exposure-based Discrimination: hosts will be more aggressive toward brood parasites with geographic overlap in populations (sympatry) compared to geographically isolated populations (allopatry).

With our new results, we discuss existing support for each hypothesis about threat discrimination in avian hosts of obligate brood parasitism, and address current gaps or biases in the literature to date that should be remedied in future studies.

## 1.3 METHODS

We searched the published literature through Google Scholar and Web of Science using the following boolean search string: “brood parasite” predator AND aggres* bird OR egg OR model OR nestling OR brood OR playback. We only included studies that met the following five criteria:

(i) studies had to experimentally test for host responses with model, playback, and/or live stimulus presentation near a host nest or on a host territory;

(ii) experiments had to compare host responses to both an obligate brood parasite and predator model within one nest stage or across multiple nest stages;

(iii) host aggression toward models had to be numerically or categorically quantified (e.g., alarm call rate, number of strikes or swoops, closest approach, aggression score) and compared between the types of models and nesting stages tested. Studies that only examined responses that were not directly related to aggression and nest defense, such as time spent foraging, were excluded;

(iv) researchers needed to provide host aggression data for one or more known stages: laying and incubating (considered "egg" stage in our review), or nestling, and data had to be provided separately for each stage tested; and, critically,

(v) studies were required to use a control model/playback in order to generate effect sizes.

For the publications focusing on multiple host species, we assessed each species tested in the study as a separate sample. For studies that presented two types of predators (adult vs. nest), we also separated the response to each type as its own sample. We labeled hosts as accepters or rejecters based on the authors' own categorization, or on other publications if not categorized by authors (e.g., Moksnes et al., 1991; Robertson & Norman, 1976); these accepter vs. rejecter labels were based on the hosts' responses to naturally laid brood parasitic eggs. This allowed us to compare studies with hosts of cowbirds and cuckoos, despite differences in egg rejection abilities between the hosts of these different lineages of brood parasites (e.g., Luro & Hauber, 2020). For example, most common cuckoo hosts are rejecters of non-mimetic (model) eggs (Stoddard & Stevens, 2010), but this may be so simply because the coevolutionary arms-race has progressed far in many of this brood parasite's host species (Stoddard & Hauber, 2017). However, these hosts often still accept the now closely mimetic cuckoo eggs at high rates when laid naturally or inserted experimentally (e.g., Hauber et al., 2015), thus we still classified those hosts behaviorally as accepters. Last, we also labeled hosts as sympatric or allopatric with the brood parasite(s) to separate studies in sympatry from allopatric samples. Studies that used hosts labeled as “unsuitable” (very rarely parasitized when in sympatry with brood parasite or brood parasite cannot survive in the nest) for parasitism were excluded.

We then formalized our meta-analysis by extracting the effect sizes for each aggression metric from published studies (Koricheva et al., 2013). To do so, we first extracted the mean, sample size, and standard deviation from the text of each study for all aggression responses to controls, brood parasites, and predators. For those studies where these data were not provided in-text, we used the R package metaDigitise (Pick et al., 2019) to digitize these values from the figures within the published papers. We calculated Hedges' G effect sizes from the mean, sample size, and standard deviation of the specific treatment group (brood parasite or predator) and the mean, sample size, and standard deviation of the control group (Hedges, 1981). As it was possible for the aggression effect sizes to be positive or negative depending on context of the aggression (e.g., a higher number of strikes and a smaller latency would both signify strong aggression), we transformed effect sizes by taking their absolute value, so that we could focus our analyses on the magnitude of the effect size.

### 1.3.1 Statistical analysis

We conducted a linear mixed model with nest stage, threat type (brood parasite vs. predator), and host type (accepter vs. rejecter) as fixed effects, and avian family as a random effect (for phylogenetic correction) to analyze whether these affected the aggression effect sizes (Hedges G) between threat models and their respective controls. This was only done with data from presentations in sympatry, as there were a number of comparisons that could not be made with allopatric data because of lack of data. To analyze whether the type of brood parasite (nestmate evictor vs. non-evictor) or predator (adult predator vs. nest predator) affected the aggression effect sizes, we conducted another linear mixed model with nest stage, specific threat type (nestmate evictor brood parasite, non-evictor brood parasite, adult predator, nest predator), and host type (accepter vs. rejecter) as fixed effects, and taxonomic family as a random effect. For our geographic overlap with brood parasites comparison (sympatry vs. allopatry) we used a subset of the data with just the brood parasite model presentations to compare responses. Hosts were determined to be in sympatry if the host population overlapped with the brood parasite species presented in each study. Our search to generate the meta-analysis dataset did not return any studies on rejecter hosts in allopatry so we could not include host type as a fixed effect. This second model had geographic overlap (sympatry or allopatry) as a fixed effect, and avian family as a random effect.

We were also interested in whether the sampling method or type of aggression measured had an influence on how aggressive hosts were toward threat presentations. For the sampling method, we categorized the studies based on the number of birds tested per stimulus sample, the number of stimuli each subject was tested with, and the number of nest stages subjects were tested across. This resulted in four sampling method categories: one bird/one stimulus/one stage, one bird/multiple stimuli/one stage, one bird/multiple stimuli/multiple stages, multiple birds/multiple stimuli/one stage.

We then ran a linear mixed model with sampling method as a fixed effect and avian family as a random effect. For type of aggression, we categorized behaviors measured as physical aggression (e.g., strikes, swoops, approach), vocal aggression (e.g., alarm calls), or combined/other response type (e.g., aggression scores, latency to respond). We ran a linear mixed model with aggression type as a fixed effect and avian family as a random effect. To determine if host type and threat type influences whether hosts favor vocal vs. physical behaviors in their defense, we ran a linear mixed model only with samples that included strictly physical or vocal aggressive behaviors, and included aggression type, threat type, and host type as fixed effects and avian family as a random effect.

## 1.4 RESULTS

Our initial boolean search yielded 1270 results, but after applying the rigorous criteria required for the meta-analytic techniques, the literature review resulted in 29 publications (see Table 1 for the full list). In total, 25 host species, 7 brood parasite species, and 17 predator species were represented across the collected studies for our review. The focal brood parasitic species for most samples were from brown-headed cowbirds, followed by common cuckoos, the two best-studied obligate brood parasites (reviewed in Davies, 2010), with most studies also occurring in the temperate zones of the Northern Hemisphere (69% of samples). The most common nest predator species used were blue jays (*Cyanocitta cristata*; 5%), common grackles (*Quiscalus quiscula*; 25%), and Eurasian sparrowhawks (*Accipiter nisus*, primarily an adult predator; 6%). Nevertheless, studies in our sample were conducted on hosts and with parasites and/or predators native to six continents - North America, South America, Europe, Asia, Africa, and Australia. We also found some biases toward three host species in our data set: yellow warblers (*Setophaga petechia*, 17% of samples), Eurasian reed warblers (*Acrocephalus scirpaceus*, 14% of samples), and red-winged blackbirds (*Agelaius* *phoeniceus*, 17% of samples). This was often the result of the same researcher(s) conducting a series of studies on a specific host-parasite interaction.

**Table 1.** Summary of avian studies that compared host aggressive responses to presentations of brood parasites and predators.

| Host species | Parasite species | Predator species | Control species | Reference |
|---|---|---|---|---|
| **American Robin (*Turdus migratorius*)** | Brown-headed Cowbird (*Molothrus ater*) | Eastern Chipmunk (*Tamias striatus*) | European Starling (*Sturnus vulgaris*) | Enos et al., 2020 |
| **American Robin (*Turdus migratorius*)** | Brown-headed Cowbird (*Molothrus ater*) | Blue Jay (*Cyanocitta cristata*), Common Grackle (*Quiscalus quiscula*) | Fox Sparrow (*Passerella iliaca*) | Sealy et al., 1998 |
| **Baltimore Oriole (*Icterus galbula*)** | Brown-headed Cowbird (*Molothrus ater*) | Common Grackle (*Quiscalus quiscula*) | Fox Sparrow (*Passerella iliaca*) | Neudorf & Sealy, 1992 |
| **Barn Swallow (*Hirundo rustica*)** | Common Cuckoo (*Cuculus canorus*) | Eurasian Sparrowhawk (*Accipiter nisus*) | Eurasian Collared Dove (*Streptopelia decaocto*), Oriental Turtledove (*Streptopelia orientalis*), Wood Pigeon (*Columba palumbus*) | Liang & Møller, 2015 |
| **Eurasian Blackcap (*Sylvia atricapilla*)** | Common Cuckoo (*Cuculus canorus*) | Eurasian Jay (*Garrulus glandarius*) | European Turtle Dove (*Streptopelia turtur*) | Požgayová et al., 2009 |
| **Carolina Wren (*Thryothorus ludovicianus*)** | Brown-headed Cowbird (*Molothrus ater*) | Blue Jay (*Cyanocitta cristata*) | Swainson's Thrush (*Catharus ustulatus*) | D'Orazio & Neudorf, 2008 |
| **Cedar Waxwing (*Bombycilla cedrorum*)** | Brown-headed Cowbird (*Molothrus ater*) | Common Grackle (*Quiscalus quiscula*) | Fox Sparrow (*Passerella iliaca*) | Neudorf & Sealy, 1992 |
| **Clay-colored Sparrows (*Spizella pallida*)** | Brown-headed Cowbird (*Molothrus ater*) | Franklin's ground squirrel (*Poliocitellus franklinii*), Common Grackle (*Quiscalus quiscula*) | Fox Sparrow (*Passerella iliaca*) | Grieef, 1995 |

**Table 1. (Cont.)**

| **Host species** | **Parasite species** | **Predator species** | **Control species** | **Reference** |
|---|---|---|---|---|
| **Common Grackle (*Quiscalus quiscula*)** | Brown-headed Cowbird (*Molothrus ater*) | Blue Jay (*Cyanocitta cristata*), Common Grackle (*Quiscalus quiscula*) | Fox Sparrow (*Passerella iliaca*) | Sealy et al., 1998 |
| **Eastern Kingbird (*Tyrannus tyrannus*)** | Brown-headed Cowbird (*Molothrus ater*) | Common Grackle (*Quiscalus quiscula*) | Fox Sparrow (*Passerella iliaca*) | Bazin & Sealy, 1993 |
| **Eastern Phoebe (*Sayornis phoebe*)** | Brown-headed Cowbird (*Molothrus ater*) | Eastern Chipmunk (*Tamias striatus*) | European Starling (*Sturnus vulgaris*) | Enos et al., 2020 |
| **Eurasian Reed Warbler (*Acrocephalus scirpaceus*)** | Common Cuckoo (*Cuculus canorus*) | Eurasian Sparrowhawk (*Accipiter nisus*) | Eurasian Teal (*Anas crecca*) and general parrot | Welbergen & Davies, 2012 |
| **Eurasian Reed Warbler (*Acrocephalus scirpaceus*)** | Common Cuckoo (*Cuculus canorus*) | Eurasian Sparrowhawk (*Accipiter nisus*) | Eurasian Collared Dove (*Streptopelia decaocto*) | Welbergen & Davies, 2011 |
| **Eurasian Reed Warbler (*Acrocephalus scirpaceus*)** | Common Cuckoo (*Cuculus canorus*) | Eurasian Magpie (*Pica pica*) | Rock Pigeon (*Columba livia*) | Campobello & Sealy, 2010 |
| **Eurasian Reed Warbler (*Acrocephalus scirpaceus*)** | Common Cuckoo (*Cuculus canorus*) | Eurasian Sparrowhawk (*Accipiter nisus*) | Eurasian Teal (*Anas crecca*) | Welbergen & Davies, 2008 |
| **Fan-tailed Gerygone (*Gerygone flavolateralis*)** | Shining Bronze-Cuckoo (*Chrysococcyx lucidus*) | New Caledonian Crow (*Corvus moneduloides*) | Common Chaffinch (*Fringilla coelebs*) | Attisano et al., 2020 |
| **Field Sparrows (*Spizella pusilla*)** | Brown-headed Cowbird (*Molothrus ater*) | Blue Jay (*Cyanocitta cristata*) | Fox Sparrow (*Passerella iliaca*) | Burhans, 2001 |
| **Gray Catbird (*Dumetella carolinensis*)** | Brown-headed Cowbird (*Molothrus ater*) | Common Grackle (*Quiscalus quiscula*) | Fox Sparrow (*Passerella iliaca*) | Neudorf & Sealy, 1992 |
| **Great Tit (*Parus major*)** | Common Cuckoo (*Cuculus canorus*) | Eurasian Sparrowhawk (*Accipiter nisus*) | Oriential Turtle Dove (*Streptopelia orientalis*) | Yu et al., 2017 |
| **Large-billed Gerygone (*Gerygone magnirostris*)** | Shining Bronze-Cuckoo (*Chrysococcyx lucidus*) | Collared Sparrowhawk (*Accipiter cirrocephalus*), Brown Goshawk (*Accipiter fasciatus*) | Willie Wagtail (*Rhipidura leucophrys*) | Noh, 2020 |

**Table 1. (Cont.)**

| Host species | Parasite species | Predator species | Control species | Reference |
|---|---|---|---|---|
| **Oriental Reed Warbler (*Acrocephalus orientalis*)** | Common Cuckoo (*Cuculus canorus*) | Eurasian Sparrowhawk (*Accipiter nisus*) | Spotted Dove (*Spilopelia chinensis*) | Li et al., 2016 |
| **Reed Parrotbill (*Paradoxornis heudei*)** | Common Cuckoo (*Cuculus canorus*) | Eurasian Sparrowhawk (*Accipiter nisus*) | Spotted Dove (*Spilopelia chinensis*) | Li et al., 2016 |
| **Red Wattlebird (*Anthochaera carunculate*)** | Pacific Koel (*Eudynamys orientalis*) | Pied Currawong (*Strepera graculina*) | Crimson Rosella (*Platycercus elegans*) | Abernathy, 2017 |
| **Red-crested Cardinal (*Paroaria coronate*)** | Shiny Cowbird (*Molothrus bonariensis*) | Guira Cuckoo (*Guira guira*) | Grayish Baywing (*Agelaioides badius*) | Segura & Reboreda, 2012 |
| **Red-winged Blackbird (*Agelaius phoeniceus*)** | Brown-headed Cowbird (*Molothrus ater*) | Blue Jay (*Cyanocitta cristata*) | Wood Thrush (*Hylocichla mustelina*) | Lawson et al., 2020a |
| **Red-winged Blackbird (*Agelaius phoeniceus*)** | Brown-headed Cowbird (*Molothrus ater*) | Common Grackle (*Quiscalus quiscula*) | Song Sparrow (*Melospiza melodia*) | Armstrong, 2002 |
| **Red-winged Blackbird (*Agelaius phoeniceus*)** | Brown-headed Cowbird (*Molothrus ater*) | Common Grackle (*Quiscalus quiscula*) | Fox Sparrow (*Passerella iliaca*) | Neudorf & Sealy, 1992 |
| **Superb Fairy Wren (*Malurus cyaneus*)** | Horsfield's Bronze Cuckoo (*Chrysococcyx basalis*) | Currawong (*Strepera*), Eastern Brown Snake (*Pseudonaja textilis*), Eurasian Sparrowhawk (*Accipiter nisus*) | Honeyeater (*Meliphagidae*) | Feeney et al., 2013 |
| **Whitehead (*Mohoua albicilla*)** | Long-tailed Cuckoo (*Urodynamis taitensis*) | Morepork Owl (*Ninox novaeseelandiae*) | Song Thrush (*Turdus philomelos*) | Lawson et al., 2020b |
| **Yellow Warbler (*Setophaga petechia*)** | Brown-headed Cowbird (*Molothrus ater*) | Blue Jay (*Cyanocitta cristata*) | Wood Thrush (*Hylocichla mustelina*) | Lawson et al., 2021a |
| **Yellow Warbler (*Setophaga petechia*)** | Brown-headed Cowbird (*Molothrus ater*) | Brown-headed Cowbird (presented eating egg) (*Molothrus ater*) | Fox Sparrow (*Passerella iliaca*) | Campobello & Sealy, 2011a |
| **Yellow Warbler (*Setophaga petechia*)** | Brown-headed Cowbird (*Molothrus ater*) | Common Grackle (*Quiscalus quiscula*) | Fox Sparrow (*Passerella iliaca*) | Gill & Sealy, 1996 |

**Table 1. (Cont.)**

| Host species | Parasite species | Predator species | Control species | Reference |
|---|---|---|---|---|
| **Yellow Warbler (*Setophaga petechia*)** | Brown-headed Cowbird (*Molothrus ater*) | Loggerhead Shrike (*Lanius ludovicianus*), Sharp-shinned Hawk (*Accipiter striatus*) | California Towhee (*Melozone crissalis*), European Starling (*Sturnus vulgaris*), Hermit Thrush (*Catharus guttatus*), Red-winged Blackbird (*Agelaius phoeniceus*), Western Meadowlark (*Sturnella neglecta*) | Kuehn et al., 2016 |
| **Yellow Warbler (*Setophaga petechia*)** | Brown-headed Cowbird (*Molothrus ater*) | Brown-headed Cowbird (presented eating egg) (*Molothrus ater*) | Fox Sparrow (*Passerella iliaca*) | Campobello & Sealy, 2011a |
| **Yellow Warbler (*Setophaga petechia*)** | Brown-headed Cowbird (*Molothrus ater*) | Common Grackle (*Quiscalus quiscula*) | Fox Sparrow (*Passerella iliaca*) | Gill & Sealy, 1996 |
| **Yellow Warbler (*Setophaga petechia*)** | Brown-headed Cowbird (*Molothrus ater*) | Gray Jay (*Perisoreus canadensis*) | Fox Sparrow (*Passerella iliaca*) | Gill & Sealy, 2004 |
| **Yellow Warbler (*Setophaga petechia*)** | Brown-headed Cowbird (*Molothrus ater*) | American Crow (*Corvus brachyrhynchos*) | House Sparrow (*Passer domesticus*) | Burgham & Picman, 1989 |
| **Yellow-headed Blackbird (*Xanthocephalus xanthocephalus*)** | Brown-headed Cowbird (*Molothrus ater*) | Blue Jay (*Cyanocitta cristata*), Common Grackle (*Quiscalus quiscula*) | Fox Sparrow (*Passerella iliaca*) | Sealy et al., 1998 |

Most studies used taxidermic models as the stimulus to evoke behaviors from hosts (92%), and the remaining studies used live stimuli (4%) or vocal playbacks (4%) exclusively (see Figure 1 for examples of setups). Due to the sizable differences in studies with these three modalities, we were unable to statistically examine the effects of stimulus sensory modality (acoustic playback vs. visual model) on host aggression toward treatments.

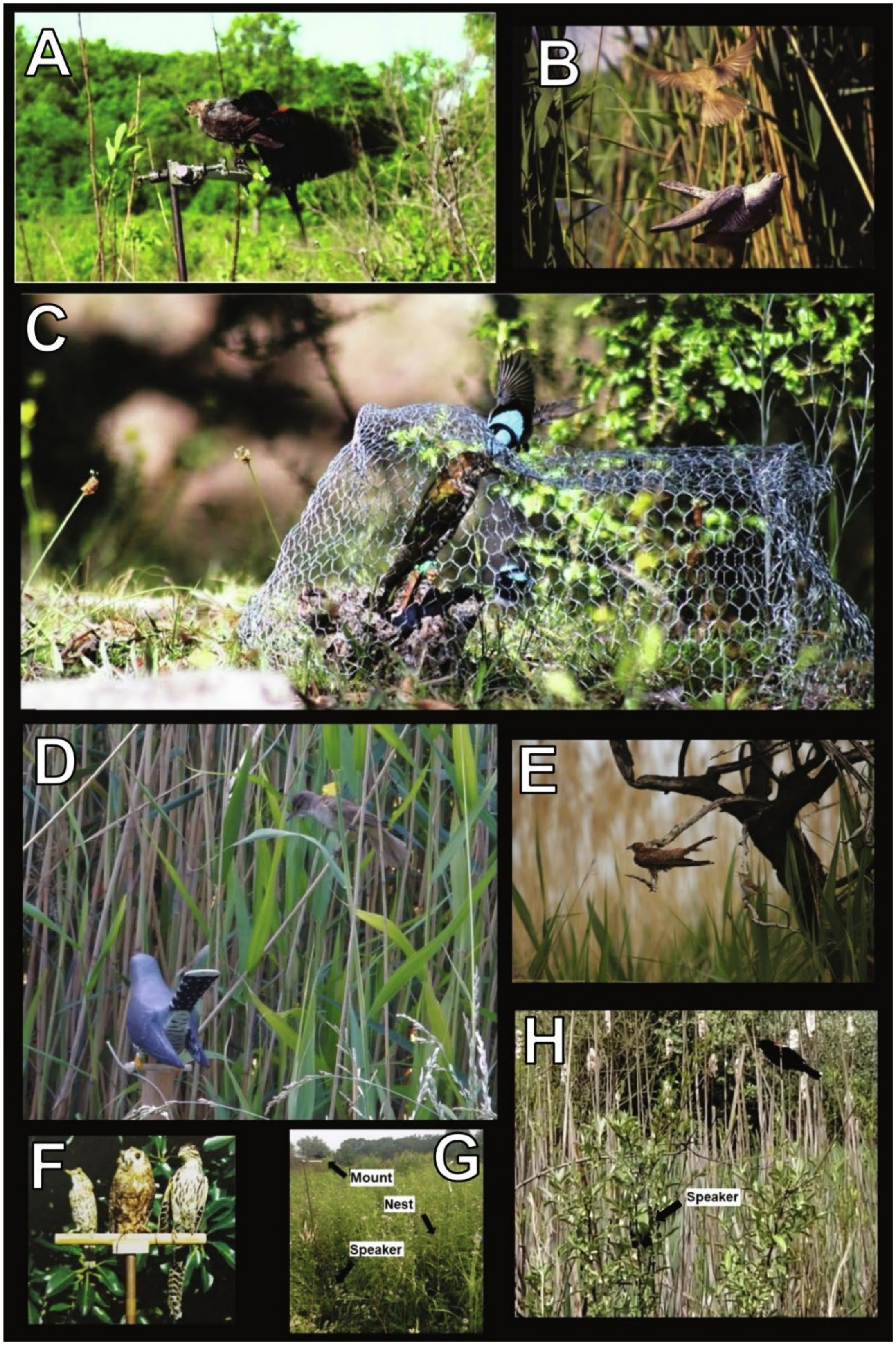

**Figure 1.** Photos of sample setups in model/playback brood parasite presentation studies. (A) A red-winged blackbird attacking a female brown-headed cowbird dummy (photo credit: K. Yasukawa),

**Figure 1. (Cont.)** (B) a great reed attacking a female common cuckoo dummy (O. Mikulica), (C) a superb fairy wren attacking a stuffed shining-bronze cuckoo dummy (W. Feeney), (D) a great reed warbler approaching a 3D printed, painted plastic common cuckoo model, (E) a sedge warbler alarm calling at a stuffed common cuckoo dummy, (F) from left to right, a song thrush (control), morepork owl (predator), and long-tailed cuckoo (brood parasite) to be presented at whitehead nests (N. Leuschner and B. Gill), (G) female brown-headed cowbird presented and its chatter called played back near an active red-winged blackbird nest (J. Lindsey), (H) a red-winged blackbird alarm calling at a speaker playing female brown-headed cowbird chatter (S. Lawson).

### 1.4.1 Host-specific and stage-specific recognition/discrimination

We found host type had a significant effect on effect size (host type term: $F_{1,391} = 4.49$, $P = 0.035$), in that accepters were more aggressive toward threat models than rejecters (Figure 2). Nest stage (stage term: $F_{1,391} = 1.96$, $P = 0.162$) and threat type (threat type term: $F_{1,391} = 0.58$, $P = 0.443$) as single terms were not significant. However, there was a significant interaction between nest stage and threat type (stage x threat type term: $F_{1,391} = 11.26$, $P < 0.001$), and between host type and threat type (host type x threat type term: $F_{1,391} = 4.69$, $P = 0.030$). There was not a significant interaction between stage and host type (stage x host type term: $F_{1,391} = 0.09$, $P = 0.761$), or between all three variables (host type x stage x threat type term: $F_{1,391} = 0.60$, $P = 0.438$). Based on post hoc pairwise comparisons, accepters were similarly aggressive to brood parasite and predator models during egg stage ($t = 0.76$, $P = 0.443$) but became significantly more aggressive toward predators compared to brood parasites during nestling stage ($t = 3.38$, $P < 0.001$), and aggressive responses overall were stronger than during egg stage ($t = 3.22$, $P = 0.001$). Accepters were specifically more aggressive than rejecters toward parasites during egg stage ($t = 2.12$, $P = 0.035$). Conversely, rejecters were more aggressive toward predators compared to brood parasites across egg ($t = 2.05$, $P = 0.040$) and nestling stages ($t = 3.47$, $P < 0.001$). Unlike accepters, rejecters did not significantly increase aggression toward predators over the nesting cycle ($t = -1.42$, $P = 0.155$). All other post hoc comparisons across nest stage or threat type were non-significant (see Supplementary Table 1).

### 1.4.2 Threat-specific recognition/discrimination

When we defined threat types more specifically (nestmate evictor vs. nest-sharer brood parasite, or adult predator vs. nest predator), we found that nest stage as a single term was significant (stage term: F1,391 = 14.87, P < 0.001), in that hosts (types: accepters and rejecters) were more aggressive to threat presentations during nestling stage compared to egg stage (Figure 3). Host type (host type term: F1,391 = 1.40, P = 0.236) and threat type (threat type term: F1,391 = 0.60, P = 0.614) as single terms were not significant in the specific threat type model. However, similar to the general threat type model (i.e., brood parasite vs. predator), there was a significant interaction between nest stage and threat type (stage x threat type term: F1,391 = 7.55, P < 0.001), and between host type and threat type (host type x threat type term: F1,391 = 4.51, P < 0.01). There was not a significant interaction between stage and host type (stage x host type term: F1,391 = 1.44, P = 0.229), or between all three variables (host type x stage x threat type term: F1,391 = 0.95, P = 0.329).

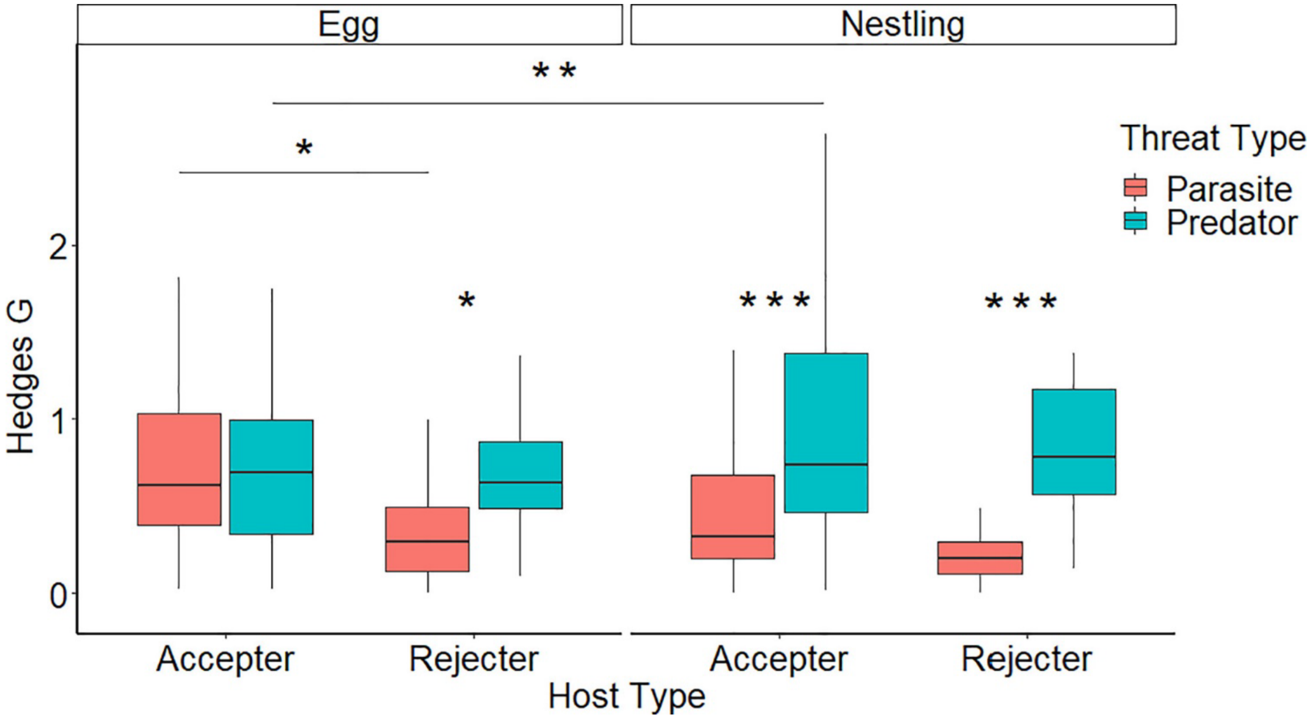

**Figure 2.** Pooled effect sizes (Hedges G) of aggression by hosts between threat types and their respective controls across studies. Effect sizes are also separated by host type and nesting stage. Box plots illustrate median values (bar), interquartile range (box), and minimum and maximum effect sizes (lines). Significant post hoc differences between groups are represented with asterisks (*** P < 0.001,** P < 0.01, * P < 0.05).

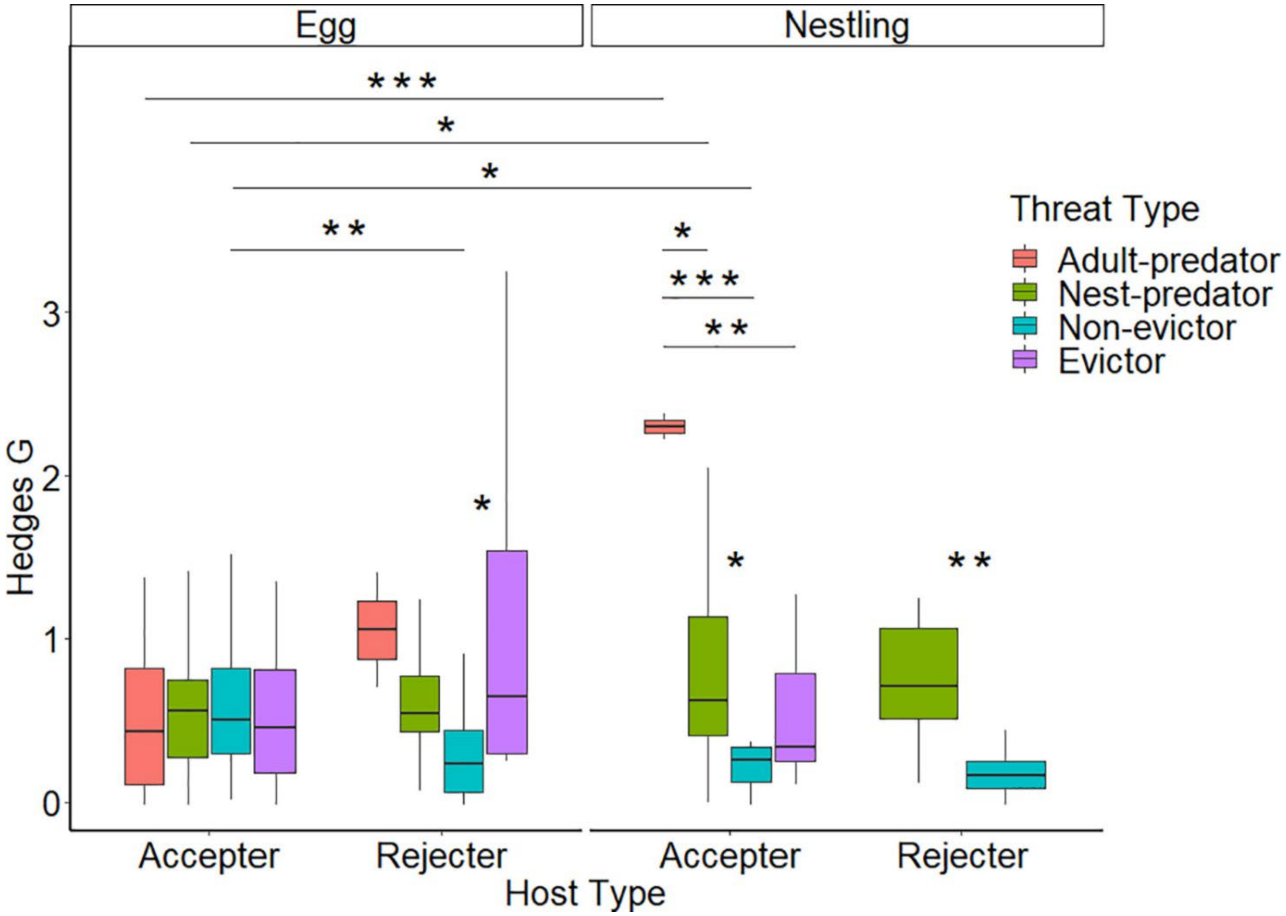

**Figure 3.** Pooled effect sizes (Hedges G) of aggression by hosts between specific threat types and their respective controls across studies. Effect sizes are also separated by host type and nesting stage. Box plots illustrate median values (bar), interquartile range (box), and minimum and maximum effect sizes (lines). Significant post hoc differences between groups are represented with asterisks (*** $P < 0.001$, ** $P < 0.01$, * $P < 0.05$).

Based on post hoc pairwise comparisons across nest stages, accepters were more aggressive toward adult ($t = 3.85$, $P < 0.001$) and nest predators ($t = 1.98$, $P = 0.048$) during the nestling stage compared to the egg stage. Accepters were more aggressive toward non-evictor brood parasites when their nests had eggs compared to nestlings ($t = 2.04$, $P = 0.041$). For evictor brood parasites, accepter aggression did not change across nest stages ($t = -0.01$, $P = 0.959$). During nestling stage, accepters were more aggressive to adult predators compared to nest predators ($t = 3.03$, $P = 0.013$), evictor brood parasites ($t = 3.49$, $P < 0.01$), and non-evictor brood parasites ($t = 4.09$, $P < 0.001$). Accepters were equally aggressive toward all threat types during egg stage, but during the nestling stage accepters were more aggressive to adult predators compared to nest predators ($t = 3.03$, $P = 0.013$), evictor brood parasites ($t = 3.49$, $P < 0.01$), and non-evictors ($t = 4.09$, $P < 0.001$). Accepters were also more aggressive to nest predators than non-evictors when they had nestlings ($t = 2.88$, $P = 0.021$).

For rejecters, the data only allowed us to compare nest predators ($t = -1.44$, $P = 0.149$) and non-evictor brood parasites ($t = 0.23$, $P = 0.817$) between nest stages, though there were no significant differences for either. Post hoc comparisons across threat types found that during the egg stage, rejecters were significantly more aggressive toward evictor brood parasites than non-evictors ($t = 2.72$, $P = 0.034$). During the nestling stage, rejecters were more aggressive toward nest predators than non-evictor brood parasites ($t = 3.47$, $P < 0.01$). Comparisons between host types found that accepters were more aggressive than rejecters toward specifically non-evictor parasites during egg stage ($t = 3.19$, $P = 0.001$). All other post hoc comparisons across nest stage, threat type, and host type were non-significant (see Supplementary Table 2).

### 1.4.3 Recognition/discrimination by geographic overlap

Our model found a significant difference in host aggression based on sympatry (geographic overlap) or allopatry (geographic isolation) between host and brood parasite populations (geography term: $F1,201 = 5.40$, $P = 0.021$; Figure 4). Specifically, hosts were more aggressive toward brood parasites in allopatry compared to sympatry with brood parasites.

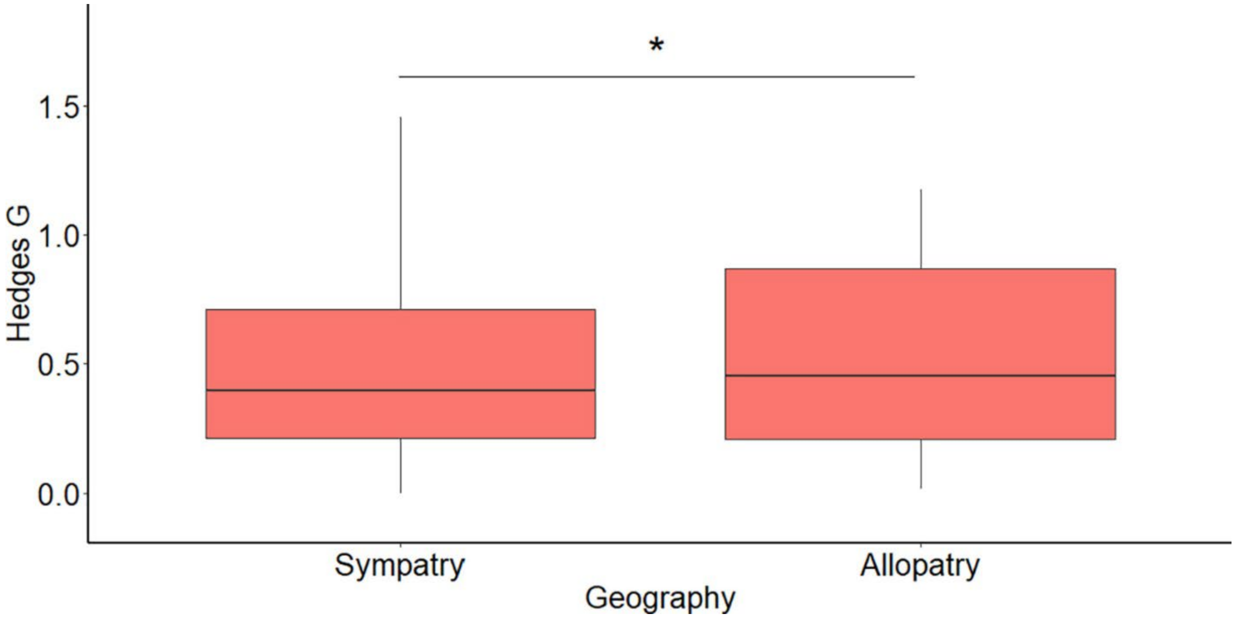

**Figure 4.** Pooled effect sizes (Hedges G) of aggression by accepter hosts between parasite models and their respective controls across studies. Effect sizes are also separated by nesting stage. Box plots illustrate median values (bar), interquartile range (box), and minimum and maximum effect sizes (lines). Significant post hoc differences between groups are represented with asterisks (* P < 0.05).

### 1.4.4 Sampling methodology and host aggression metrics

Type of sampling method used (single or multiple birds tested, single or multiple stimuli used or nest stages tested) did not significantly influence effect sizes (F1,417 = 0.77, P = 0.567). However, effect sizes were significantly different based on the type of behavioral aggression measured (aggression term: F1,417 = 12.83, P < 0.001; Figure 5). We found that behaviors that included both vocal and physical components had higher effect sizes than vocal (t = 3.95, P < 0.001) or physical (t = 5.09, P < 0.001) behaviors alone. We also examined whether host type and threat type influenced whether hosts use vocal or physical behaviors more frequently in their defense. We did not find any significant interactions between aggression type and threat type (aggression x threat type term: F1,417 = 1.88, P = 0.170) or between aggression type and host type (aggression x host type term: F1,417 = 2.35, P = 0.125).

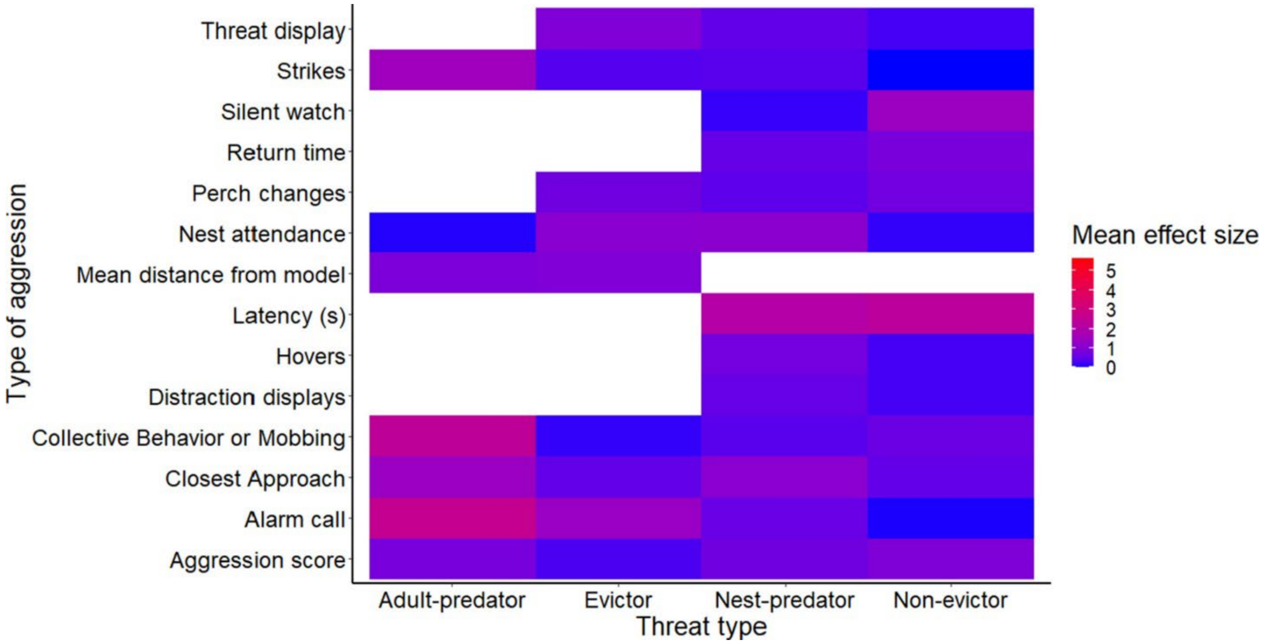

**Figure 5.** A heat map describing patterns of the mean effect sizes for each threat type based on the type of aggression measured. Lighter colors (red) show an effect size with a greater magnitude. The areas with an absence of color on the plot represent gaps in the collected literature where no studies currently exist.

## 1.5 DISCUSSION

### 1.5.1 Host-specific and stage-specific recognition/discrimination

Overall we found that both host types and nest stage influence the effect sizes of aggression by parental birds in response to brood parasitic or predatory intruders at the nest (Figure 6). Accepters were more aggressive than rejecters to threats, likely because of the differences in their responses to brood parasites. Accepters were equally aggressive to brood parasite and predator presentations when they had eggs, but compared to brood parasites, accepters became significantly more aggressive toward predators during the nestling stage. Conversely, rejecters were consistently more aggressive toward predators compared to brood parasites across nest stages.

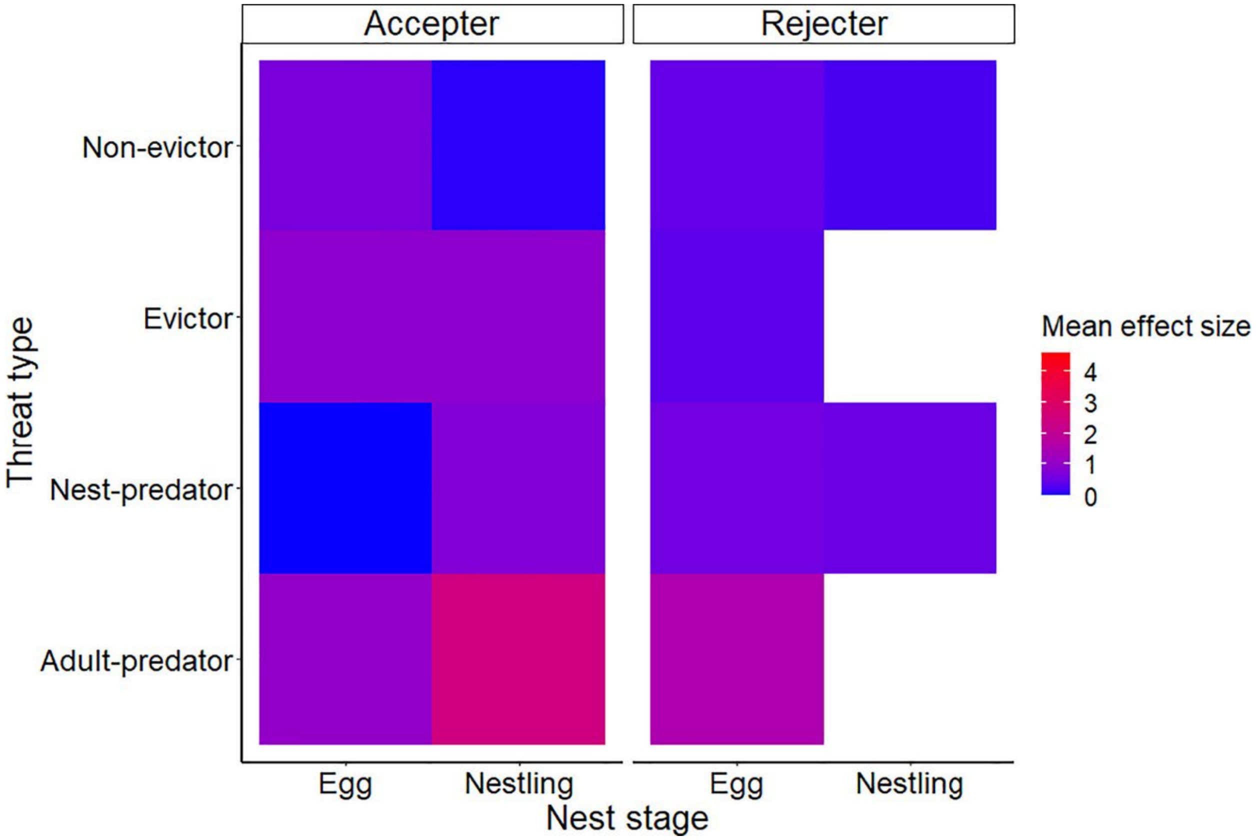

**Figure 6.** A heat map describing patterns of the mean effect sizes for three focal comparisons: threat type (brood parasite vs. predator), host type (accepter vs. rejecter), and nesting stage (egg vs. nestling). Lighter colors (red) show an effect size with a greater magnitude. The areas with an absence of color on the plot represent gaps in the collected literature where no studies currently exist.

Taken together, our results found patterns consistent with our predictions, in that accepters respond aggressively toward brood parasites predominantly during laying and incubation stages when brood parasitism poses the highest risk. In contrast, rejecters, which rely on egg discrimination rather than front-loaded defenses, do not respond as aggressively to brood parasites during either nest stage. Furthermore, parents are expected to invest more energy into defending their young from predatory threats with increased investment in offspring, as well as parental assurance in the survival of the offspring (Montgomerie & Weatherhead, 1988; Regelmann & Curio, 1983). The patterns we found support this, as host aggression toward predators either remained high across nest stages, as was the case for rejecters, or increased with nest age as we observed for accepters.

### 1.5.2 Theat-specific recognition/discrimination

#### 1.5.2.1 Brood parasite type

Brood parasites were classified as evictor parasites, which eject or kill nestmates, or non-evictor parasites, which share the nest, and may even benefit from host nestlings in it (Hauber, 2003, Kilner et al., 2004; Winnicki et al., 2021). For accepters, we found, against our prediction, that aggression levels toward evictor and non-evictor brood parasites were the same, regardless of nest stage. Though non-evictor nestlings are considered less costly to fitness than evictor nestlings (Kilner, 2005), encounters with adult non-evictors may be costly, which model presentation experiments simulate. For example, non-evicting adult brown-headed cowbirds can cause total nest failure by depredating non-parasitized nests to regulate the timing of their parasitism opportunities (referred to as “farming;” Arcese et al., 1996; Clotfelter & Yasukawa, 1999). In this case, hosts may instead recognize non-evicting brood parasites as nest predators, with continued high aggression toward brood parasites during the nestling stage. For accepter species, discriminating between reproductive threats may thus depend on personal experience with the brood parasite more so than the brood parasite's reproductive strategy (nestmate evicting vs. non-evicting). Yellow warblers, for example, are more aggressive toward brown-headed cowbirds when they experience the brood parasite as an egg predator (Campobello & Sealy, 2011a). Additional studies on other accepter hosts and brood parasite types (e.g., Campobello & Sealy, 2011b) are necessary to further explore the effect of personal experience on enemy recognition.

Rejecters responded with consistently low levels of aggression toward non-evictors across nest stages, as predicted. However, rejecters exhibited more aggression overall toward evictors than non-evictors, despite little risk of parasitism from either. This result may be an artifact of the small sample size in our dataset representing rejecter host species with an evictor brood parasite (n = 4 samples).

Additionally, only three rejecter hosts - great tits (*Parus major*), reed parrotbills (*Paradoxornis heudei*), and Eurasian blackcaps (*Sylvia atricapilla*) - are represented in these samples, two of which are parasitized at lower rates (great tits and reed parrotbills; Li et al., 2016; Liang et al., 2016). Moreover, great tits and Eurasian blackcaps are considered generally aggressive across ecological contexts (great tits: Lang & Leimer, 2001; Samplonius, 2018; Eurasian blackcaps: Darolová et al., 2020; Morganti et al., 2017). As such, aggression responses we observed among rejecters may be related to species-specific baseline aggression levels, and not related to brood parasite type per se.

#### 1.5.2.2 Predator type

We classified predators as either nest predators, which are strictly threats to reproductive success, or adult predators, which hosts recognize as direct threats to their own survival (e.g., genus Accipiter hawks and "small" owls; Congdon et al., 2020; Sieving et al., 2010). There were no significant differences in aggression toward predator types (compared to each other and to parasite types) during the egg stage. In contrast, during the nestling stage hosts were more aggressive toward adult predators compared to other threats (nest predators, evictor brood parasites, non-evictor brood parasites). Notably, adult predation risk literature suggests that birds often invest in their own survival over reproductive effort when perceived adult predation risk is high (made apparent by models or playbacks of adult predators at nests; LaManna & Martin, 2016; Oteyza et al., 2021; Zanette et al., 2011). However, short-lived species are more likely than long-lived species to defend nests against adult predators, because of the high premium placed on annual reproductive output in short-lived species (e.g., Oteyza et al., 2021). All host species included in this specific analysis are considered short-lived or "fast" life-history species (n = 9 species, see Table 1). Our results could thus reflect tradeoffs that occur between survival and reproduction as nests approach fledging, with parents highly invested in reproductive success willing to defend nests against adult predators despite potential risks to survival.

During the nestling stage, hosts responded significantly more aggressively toward nest predators than non-evictor brood parasites, but not toward evictors. Responses toward non-evictor brood parasites during the nestling stage support our prediction, but responses toward evictor brood parasites do not. Why respond with equal aggression levels toward nest predators and any type of brood parasite, if the latter is only a threat while hosts are laying and incubating? Curiously, most studies evaluating host responses to evictor parasites used common cuckoo models, a species that superficially resembles an adult predator, the Eurasian sparrowhawk *Accipiter nisus* (Ma et al., 2018 and references therein). It is possible that in these cases, hosts instead perceived brood parasite models as adult predators and responded aggressively due to high reproductive investment during the nestling stage (despite potential survival risks, discussed above). Some *Cuculidae* species can also be nest predators themselves, and as such they may still pose a risk to reproductive investment even during the nestling stage (Gill et al., 2018; Zahavi, 1979).

### 1.5.2.3 Recognition/discrimination by geographic overlap

We found that hosts in geographic isolation from brood parasites (i.e., allopatric) were significantly more aggressive toward the brood parasite presentations compared to sympatric populations. Our result, however, only provide limited support for the hypothesis that allopatric host populations no longer recognize brood parasites as a unique threat (or nest threat at all; sensu Briskie et al., 1992; Kuehn et al., 2016). Our small sample size of allopatric studies for this analysis (n = 6) precluded us from addressing two important issues. First, we could not consider the effect of nest stage on allopatric host aggression levels, which as demonstrated in this review is crucial to address host discrimination between brood parasites and nest predators. Indeed, only one study was conducted in allopatry during the nestling stage (Lawson et al., 2020b). Second, we could not compare allopatric host aggression between accepter and rejecter hosts, as there were no studies conducted on rejecter host populations that were allopatric from brood parasites. Future experiments evaluating loss of enemy recognition in host species should aim to include more host species and conduct exposure experiments throughout the nesting cycle.

## 1.6 RESEARCH NEEDS AND FUTURE DIRECTIONS

To date, most studies have addressed enemy recognition by accepter hosts in sympatry with their non-evicting brood parasites (Figure 7). Compared to hosts of non-evictors, fewer studies address enemy recognition by hosts of evictor brood parasites. Enemy recognition by rejecter hosts of evictor parasites has received the least attention, with studies solely testing aggressive responses during the egg stage in sympatric populations. Our literature review also revealed two knowledge gaps in the literature on host enemy recognition and nest threat discrimination. Currently, too few studies address whether (1) rejecter hosts of evictor parasites discriminate between nest threats during the nestling stage, specifically when populations are sympatric and (2) accepter hosts of non-evictor brood parasites still discriminate between nest threats during the nestling stage when a host population is allopatric from the brood parasite.

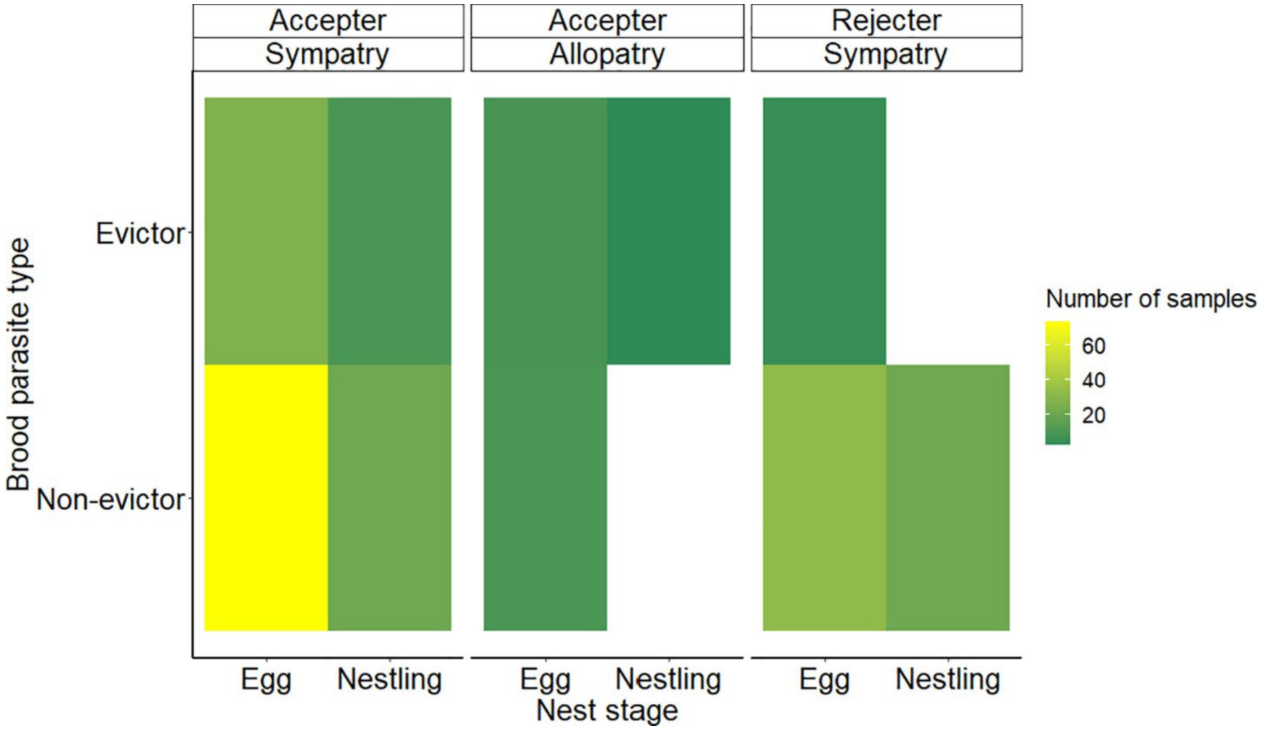

**Figure 7.** A heat map describing bias of samples in our meta-analysis dataset on enemy recognition (brood parasite vs. predator) based on four focal comparisons: brood parasite type (evictor vs. non-evictor), host type (accepter vs. rejecter), nesting stage (egg vs. nestling), and geographical overlap (sympatry vs. allopatry). Lighter colors (yellow) show an effect size with a greater magnitude. The areas with an absence of color on the plot represent gaps in the collected literature where no studies currently exist. Our meta-analysis did not contain any studies with rejecter hosts tested in allopatry so this column was omitted.

Although a relatively rare reproductive strategy, brood parasitism occurs in diverse orders of birds and involves diverse orders of host species. Our analyses only included hosts of brood parasites from Passeriformes (i.e., passerine birds), whereas brood parasites primarily were cuckoos (Cuculiformes: *Cuculidae*) and cowbirds (*Icteridae*). All passerine hosts in our review have similar life-history traits: most are open-cup nesting species (but see Liang et al., 2016 for a cavity-nester) and all have altricial young that require intensive parental care. However, our literature review does not include parasitic finches (*Viduidae*; i.e., Feeney et al., 2015) or brood parasitic species from other taxonomic groups with different life-history traits, such as honeyguides (Piciformes: *Indicatoridae*) and the only brood parasitic waterfowl species (Anseriformes, black-headed duck, *Heteronetta atricapilla*; Lyon &

Eadie, 2013). Honeyguides, for example, are nestmate-killing parasites and primarily parasitize cavity-nesting hosts, which generally experience low predation risk compared to open-cup nesters (Spottiswoode, 2013). Honeyguide hosts, which are primarily woodpeckers and allies (Piciformes) and bee-eaters and allies (Coraciiformes), may thus perceive brood parasitism and nest predation risk differently than their open cup-nesting counters with non-evicting brood parasites. The black-headed duck is unique in that offspring are precocial and take care of themselves soon after hatch day (Cabrera et al., 2017), relieving host parents of any cost to brood parasitism. Several waterfowl species recognize both facultative brood parasites and predators as nest threats (Sorenson, 1997), but it is unknown if hosts treat the black-headed duck as a nest threat or a non-threatening heterospecific intruder. Testing hosts from these taxa with diverse life-history traits will greatly advance our understanding of enemy recognition and associated nest defense by avian hosts.

We also found a clear bias of host focal species, with the yellow warbler, red-winged blackbird, and Eurasian reed warbler being the most commonly tested hosts. These species have specialized nest defense behaviors that are not necessarily broadly applicable to other host species, therefore limiting our ability to generalize our findings across the avian taxa. Yellow warblers, for example, use referential alarm calls to specifically inform their mates of brown-headed cowbirds at the nest, which elicits a cowbird-specific defense of “sitting tightly” on the nest (Gill & Sealy, 1996; Gill & Sealy, 2004; Lawson et al., 2021a). Red-winged blackbirds and Eurasian reed warblers use social information from neighbors to assess brood parasitism risk and adjust their aggression levels accordingly (Campobello & Sealy, 2011b, Lawson et al., 2020a), but it is unknown how widespread social information use is for nest defense purposes in other host lineages (but see: Feeney & Langmore, 2013). Future studies should experiment with other common hosts to determine if our findings are broadly indicative of host discrimination, or strictly indicative of those with unique, adaptive behaviors to combat brood parasitism with (such as referential alarm calling and social information use).

Almost all studies that met our inclusion criteria used taxidermic and/or artificial models as a visual stimulus in their presentations at nests. Vocal playbacks are also used as a brood parasite or predator stimulus in the literature (e.g., Lawson et al., 2020a, Lawson et al., 2021a) yet only two such studies using vocal playbacks as the sole stimulus fit the criteria described above. In turn, we did not find any significant differences between physical and vocal aggression depending on host types or type of threat presented. Still, the sensory modality of the stimulus presented, whether visual, acoustic, or even olfactory (Soler et al., 2014), could affect the type of behaviors and/or magnitude of responses that hosts include in their nest defense repertoires (Duckworth, 1991; Grieef, 1995). Thus, we suggest future studies that compare aggressive responses of hosts to brood parasites and predators expand to using acoustic playbacks and other sensory forms of stimuli to simulate nest threats to hosts.

Another noteworthy observation is that there is considerable bias in which brood parasite type is paired with which predator type in the literature collected. Specifically, the majority of studies in our collection paired non-evictor brood parasites with nest predators (54%), or evictor brood parasites with adult predators (29%). Our findings show that certain pairings are associated with higher effect sizes between treatments, suggesting that researchers may draw different conclusions from their study depending on which pairing they had decided on for experimentation. With the potential for hosts to respond differently depending on the brood parasite and the predator models used, future studies should utilize multiple brood parasite-predator dyads to diversify the models used to evoke aggression responses. Additionally, future studies should diversify the aggressive behaviors by hosts that are measured. Our results suggest that measures that incorporate both physical and vocal responses by hosts are associated with larger effect sizes and may be better at teasing apart differences in response between threat models and controls. However, we caution about the use of combined aggression scores in particular, as these scores can be subject to biases which may exaggerate differences between groups.

We found that there was also a bias in sampling methodology across studies in our dataset, where most presented multiple stimuli to the same subject (individual, nest, or territory), and often across multiple nest stages, leading to possible habituation or sensitization by focal hosts. However, we found that sampling method type did not bias effect size data, suggesting that robust responses to models by host populations can be consistently measured across contexts without confounds due to the sampling paradigm. Still, it should be noted that studies included herein effectively randomized models to reduce bias and avoided pseudoreplication of results both on an individual and temporal basis. We suggest that future presentation experiments with brood parasite vs. predator models continue to make these efforts to minimize pseudoreplication (e.g., Kroodsma et al., 2001).

## 1.7 CONCLUSIONS

1. Enemy recognition by avian host species depends on the magnitude of the threat to reproductive success, which is shaped by specific traits of the host (accepter vs. rejecter), the brood parasite (nestmate-evictor vs. nest-sharer), the predator (adult vs. nest predator), and how predictably hosts are exposed to brood parasitic threats (sympatry vs. allopatry).

2. Host discrimination between reproductive threats is likely an adaptive behavior, and may be widespread among all avian lineages experiencing different types of nest threats, including brood

parasitism, nest predation, or other (such as highly aggressive conspecific or heterospecific competitors).

3. Future studies addressing the knowledge gaps identified here, or overlaying additional factors that influence discrimination capabilities (such as personal vs. social information use or life-history traits) will greatly improve our understanding of avian enemy recognition and its payoffs to reproductive success.

# CHAPTER 2: PAIRING STATUS MODERATES BOTH THE PRODUCTION OF AND RESPONSES TO ANTI-PARASITIC REFERENTIAL ALARM CALLS IN MALE YELLOW WARBLERS

## 2.1 ABSTRACT

Defending offspring incurs temporal and energetic costs and can be dangerous for the parents. Accordingly, the intensity of this costly behavior should reflect the perceived risk to the reproductive output. When facing costly brood parasitism by brown-headed cowbirds (*Molothrus ater*), where cowbirds lay eggs in heterospecific nests and cause the hosts to care for their young, yellow warblers (*Setophaga petechia*) use referential "seet" calls to warn their mates of the parasitic danger. Yellow warblers of both sexes produce this call only in response to cowbirds or seet-calling conspecifics. Seet calls are mainly produced during the laying and incubation stages of breeding, when risk of brood parasitism is highest, rather than during the nestling stage. On the other hand, general alarm calls (chips) are produced throughout the nesting cycle and are also used in conspecific interactions unrelated to nesting. We hypothesized that context shapes responses prior to breeding as well, such that yellow warblers without a mate and active nest would be less likely to respond to playbacks that simulate brood parasitism risk. To test this hypothesis, we presented playbacks of two nest threats, cowbirds (brood parasite) and blue jays (*Cyanocitta cristata*; nest predator), on territories of unmated male warblers (unpaired) and male warblers with a known mate (paired). We found that unpaired males were unresponsive toward playbacks indicating nest threats, whereas paired males were significantly more aggressive and vocal toward these playbacks compared to control playbacks. However, both paired and unpaired males were vocally responsive toward chip calls, which are informative for males regardless of pairing status. Male yellow warblers appear to adjust their responses during the earliest stages of breeding depending on the contextual relevance of specific

threat stimuli, and together with prior studies, our work further supports that referential seet calls are associated with stage-specific risk of brood parasitism.

## 2.2 INTRODUCTION

For many organisms, parents often spend time and energy defending their offspring from various risks, such as predators, to enhance reproductive success. Such defenses can be costly and potentially dangerous for the parents, as individuals caring for offspring are often in poorer body condition, have lower survival rates, and experience shorter life spans compared to non-breeding individuals or less attentive parents (reviewed by Alonso-Alvarez & Velando, 2012). To mitigate these costs, individuals should only spend time and energy on aggression toward threats to their reproductive investment if they have any offspring to protect in the first place. Moreover, selection should favor parents that are able to discriminate between different types of threats to offspring and then respond with appropriate defensive behaviors, which are matched to the potential value of their reproductive investment.

Context-specific defensive alarm calls are widespread across taxa (for review see: Blumstein, 1999b; Evans & Evans, 2007; Gill & Bierema, 2013; Hollé & Radford, 2009; Manser et al., 2002) and often encode predator type and risk. Because predators differ in hunting style and threat, these taxa produce distinct calls and defensive responses in response to specific predators. Moreover, context-specific calls elicit defensive responses appropriate for the specific predator. Among birds, for example, many species face multiple types of nest threats, with each incurring different reproductive costs for the parents (Davies, 2010; Martin, 1992). Nest predators, for example, often lead to complete reproductive failure by consuming all eggs and/or nestlings in the nest (Martin, 1992). In contrast, obligate brood parasites, which lay their eggs in other species' nests and impose the care of the foreign young upon the host (Davies, 2010), reduce chances of nestling survival as parasitic nestlings often eliminate or otherwise outcompete host nestlings for parental provisions (Hauber, 2003; Kilner, 2003). Given the differences among types of risk that the various threats pose to reproductive success, parents should modulate their responses and use threat-specific behaviors when defending the nest.

Many host species use both private and social information to assess imminent risk to their nests, the latter of which occurs often by eavesdropping on vocalizations by the threats themselves or the alarm calls of conspecifics (Gill et al., 1997a; Gill & Sealy, 2003; Henger & Hauber, 2014; Lawson et al., 2020a; Moksnes et al., 1991). Accordingly, hosts of brood parasites have been shown to be able to discriminate between different nest threats (i.e., parasites vs. nest predators) based on their vocalizations, and adjust their defense behaviors accordingly. For example, some hosts respond most aggressively toward playbacks of brood parasites during laying/incubation, when nests are most vulnerable to brood parasitism (Fiorini et al., 2009; Geltsch et al., 2016), whereas aggression toward nest predators typically intensifies during the nestling stage, when offspring are more valuable in terms of cumulative reproductive investment (Burgham & Picman, 1989; Campobello & Sealy, 2010; Duckworth, 1991; Neudorf & Sealy, 1992). Moreover, hosts can eavesdrop on their neighbors' alarm-calling behavior to collect social information about the type of nest threat present, then adjust their behavior to match the threat at hand (e.g., Gill & Sealy, 2003; Lawson et al., 2020a).

The value of social information revealing risk to the nest should depend on breeding status and stage. As such, individuals residing in breeding habitat but without a mate or nest (i.e., non-breeders) should not respond as aggressively to social cues revealing nest risk, specifically nest predation and brood parasitism cues. Doing so could take time and energy away from more relevant reproductive tasks of defending territories and attracting mates (sensu Rieucau & Giraldeau, 2011). To date, however, few playback experiments have explored the hypothesis that non-breeders value social information less than breeders do.

The yellow warbler (*Setophaga petechia*) is an exceptional species to test this hypothesis because of its unique alarm-calling behavior and nest defense system. Yellow warblers are a common host of obligate brood parasitic brown-headed cowbirds (*Molothrus ater*; hereafter "cowbird") and are the only North American species known to have evolved a referential "seet" call that specifically informs their mates of the presence of parasitic cowbirds (Gill & Sealy, 2004; Hobson & Sealy, 1989a). Functionally referential alarm calls are unique in that they are structurally different from other calls, they elicit specific behavioral responses from listeners, and are only produced in specific contexts (Evans et al., 1993; see Gill & Bierema, 2013 for review). As such, the seet call is primarily produced in response to the presence of female cowbirds, their calls, or other yellow warblers' seet calling (Gill et al., 1997a, 2008; Gill & Sealy, 2004), and specifically during the laying and incubation nest stages (Gill et al., 2008; Hobson & Sealy, 1989a) when the nest is at the highest risk of successful brood parasitism (Fiorini et al., 2009). While both male and female warblers seet, only female warblers return to and "sit tightly" on their nest after hearing the seet call, as if to physically interfere with the process of laying a brood parasitic egg (Gill et al., 1997a; Hobson & Sealy, 1989a; Kuehn et al., 2016). Yellow warblers also have a "chip" call used to warn mates of nearby predators, as well as during conspecific interactions, including male–male territorial defense (Lowther et al., 1999). Past experiments have demonstrated context-dependent responses by male warblers to seet calls based on nest stage (incubation vs. nestling; Gill & Sealy, 2003, 2004). This response has not been tested with pairing status, which should also affect the contextual importance of the seet call as the value of information likely varies with pairing status (discussed above). Because of the unambiguous link between seet calls and nest defense against brood parasites, we expect that only paired yellow warblers with nest investments benefit from the social information available in seet calls, while all yellow warblers should respond to chip calls, as these vocalizations are not related solely to nest defense (Gill & Sealy, 2003; Hobson & Sealy, 1989a).

We designed a playback experiment to test the hypothesis that yellow warblers modulate their responses to social information about nest-related threats, specifically brood parasitism or nest predation, depending on pairing status (and therefore nest investment). We presented playbacks of nest threats to males holding territories but without mates or nests (hereafter: unpaired), and to paired males with mates and active nests (hereafter: paired). We predicted that unpaired males, without current investment in offspring, would respond similarly to playbacks of cowbird chatters, yellow warbler seet calls, and nest predator vocalizations compared to playbacks of control vocalizations and that paired males would respond more aggressively than unpaired males to all non-control treatments. In turn, we also predicted that both unpaired and paired males would respond equally to playbacks of chip calls due to the relevance of the social information that they transmit. While it may seem likely that non-breeding birds would not respond to calls denoting nest threats, little direct empirical evidence exists. Furthermore, by testing these early stage responses, we can begin to understand at what point in the nesting cycle males mobilize aggression toward specific threats to their own reproductive investment.

## 2.3 METHODS

### 2.3.1 Sites and study species

The experiment was conducted at multiple sites in Champaign (n = 3), Iroquois (n = 1), and Vermillion counties (n = 3) in central Illinois, USA, where yellow warblers are common hosts of cowbird parasitism (Kelly et al., 2019; Lawson et al., 2020a; Merrill et al., 2017). Sites were comprised of mesic shrubland habitat, with dominant shrubs including willow (Salix spp.), dogwood (Cornus spp.), and autumn olive (Elaeagnus umbellate), with mesic grasses abundant among shrubs (Kelly, 2017; Kelly et al., 2018; Lawson et al., 2020a). Yellow warblers arrive at our sites in late April and breed from early May through late June, with a peak and synchronous period of breeding during mid-to-late May (Kelly et al., 2019; Lawson et al., 2020a). In this region, parasitism rates range from 30% to 40% (pers. obs., Merrill et al., 2017).

### 2.3.2 Determining pairing status of males

We conducted playback trials on active yellow warbler territories of paired and unpaired males from mid-May to late June in 2018 and 2019. We first systematically visited each site, 2–3 times a week spaced 1–2 days apart, to search for actively singing males and to determine pairing status. During visits, we followed each male at the site for 30 min on at least two separate, sequential trips and noted any mate-guarding, a behavior commonly used to assign pairing status in Parulidae warblers, where males closely follow their mate while she is laying (e.g., yellow warblers: Hobson & Sealy, 1989b; other Parulidae: Chuang-Dobbs et al., 2001; Stutchbury et al., 1994; Toms, 2012). We also searched male territories (within 30 m of male's song perch, the average size of a yellow warbler territory in central Illinois; Kelly et al., 2018) for active nests and/or a females exhibiting nest defense behaviors (alarm-calling, perch-switching, wing-flicking, circle-flight), which have been used to assign pairing status in similar studies (Ficken & Ficken, 1965; Hobson & Sealy, 1989b; Marshall & Balda, 1974; Mitra, 1999).

Males were considered “unpaired” if, after one week of visits, no female was seen on the territory and no nest was found. We considered a male “paired” if we observed mate-guarding and/or evidence of an active nest during the week of observation. Paired males were only tested if their nest was in the laying or incubation stage, when yellow warblers are most likely to give seet calls (Gill et al., 2008; Gill & Sealy, 1996). We verified nest stage prior to trials by checking the nest to confirm its content, or if the nest location was unknown, by observing males and females for signs of incubation/laying and specifically not of other nesting stages (e.g., carrying nesting material for building or delivering food for nestlings).

It is important to note that males were not banded, and we assumed that unbanded males did not abandon their territories between site revisits. It is nonetheless possible that in some cases we still assessed two different males during visits when determining pairing status. For paired males, we consider this unlikely, because actively breeding males remain faithful to their territories for several weeks (Kelly et al., 2018). Unpaired males can abandon territories; however, if abandonment occurred within the short time window between playbacks, then it is likely that any other male encountered and tested on our second visit would have also been unpaired and with no active nest (Weary et al., 1994). We, therefore, do not consider that any potential movement of unbanded individuals confounded our treatments or precluded us from assigning pairing status.

### 2.3.3 Playback stimuli construction

Playback files and methodology for this experiment follow those from another playback experiment conducted in the same yellow warbler population but focused on heterospecific eavesdropping by red-winged blackbirds (*Agelaius phoeniceus*; Lawson et al., 2020a). In short, we created exemplars for five different playback treatments: (a) female cowbird chatters (brood parasite), (b) yellow warbler seet calls (cowbird-specific anti-parasitic alarm call), (c) yellow warbler chip calls (general alarm call given toward nest threats and during conspecific interactions; Gill & Sealy, 1996; Hobson

& Sealy, 1989a), (d) blue jay calls (*Cyanocitta cristata*, a nest predator; Smith et al. 2013), and (e) wood thrush songs (*Hylocichla mustelina*, an innocuous sympatric heterospecific control ; Kelly et al., 2019, Lawson et al., 2020a).

Audio files were obtained from Xeno-Canto, all sourced from the Midwestern United States (Illinois, Michigan, Minnesota, and Ohio), except for seet calls, which were sourced from Manitoba, Canada, via Gill and Sealy (2003). Exemplars were created using Adobe Audition CC 2019. To minimize signal-to-noise ratio in playback files, frequencies below 500 Hz, which are well below the range of any of our stimuli, were filtered out. Vocalizations from at least three different individuals comprised each exemplar. To further reduce pseudoreplication (Kroodsma et al., 2001), we constructed five different exemplars per playback treatment, and chose one exemplar file randomly for each trial (described below). Individual vocalizations were placed in a random order and then repeated to create the 10-min exemplar. Intervals of silence were placed between vocalizations, ranging from two to six seconds based on rates found in natural recordings on Xeno-Canto. Playbacks were broadcast at ~90 dB (measured 0.5 m from speaker with a Leaton L815 digital sound level meter).

Playbacks were presented from an AYL-SoundFit speaker connected to a Samsung Galaxy 8 cellular phone with the audio files. We placed the speaker ~1 m high in vegetation and recorded data from >10 m away. If a nest location was known, the speaker was placed 5–6 m from the nest; otherwise, the playback speaker was placed 5–6 m from the yellow warbler male's most commonly seen song post. The playbacks lasted for 10 min/trial. Each yellow warbler territory was retested two-to-five times 24–72 hr later (mean = 45.6 hr) to avoid habituation, with a different, randomly assigned treatment to minimize order effects.

### 2.3.4 Playback experiment

We conducted playback trials on target males after pairing status was determined. Playbacks trials occurred between 5:00 and 12:00 hr local time. Because we did not band territorial birds for individual identification, we only tested nests that were ≥ 30 m apart to maintain independence. Nests at this distance likely belonged to different breeding birds based on average territory size of yellow warblers (DellaSala, 1986; Kendeigh, 1941), including those breeding in Illinois (Kelly et al., 2018). We also waited 30–60 min between playbacks at neighboring sites to avoid any carryover effects on individuals in the proximity (Lawson et al., 2020a).

Each yellow warbler received two of five different playback treatments on separate days. If unpaired males became paired by the second trial (as evidenced by female presence and mate-guarding behavior described above), males were counted as paired for the second trial only ($n = 1$). During the playback trials, we recorded the following behavioral responses from paired and unpaired male warblers within 30 m of the speaker: (a) response latency (sec after the start of trial when a switch to aggressive behaviors occurred: posturing, hopping, alarm calling, or attacking the speaker); (b) total number of seets; (c) total number of chips; and (d) closest approach to the speaker (m). Five paired males (all in 2019) could not be retested with a second playback because their nests were depredated between trials. Likewise, 11 unpaired males were not retested ($n = 4$ in 2018, $n = 7$ in 2019) because the male was no longer present on the territory for the second trial.

### 2.3.5 Statistical analyses

We evaluated whether playback treatment affected the four response variables of interest (latency, number of seet calls, number of chip calls, and closest approach) using separate linear models for each. All models included playback treatment, pairing status, date (ordinal days after start of season—May 1st) and a treatment by pairing status interaction as fixed effects. For latency, seet call, and closest approach data, we ran mixed models with year (2018 or 2019) and trial order (to account for repeated playbacks at the same site) as random effects. For the seet call model, we included year and trial order as predictors because the random effect model did not converge. For latency and seet data, there were a large number of non-responses (bird never responded or never seet called). As such, both variables were analyzed using separate zero-inflated negative binomial models, which first analyses the data as a binomial variable (response vs no response) to determine whether non-responses were more common depending on treatment or pairing status. We then separately analyzed only data where males had responded (<600 sec latency or seet called) to determine whether the magnitude of the responses (seconds of latency or number of seet calls) varied between treatments or pairing status. For the second model (responses only), we square-root transformed the latency data to obtain a normal distribution. The seet data were analyzed with a Poisson fit. For the chip call analysis, we log transformed the data to reduce variance, and fitted the data with a Poisson distribution to run a general linear model. Last, we square-root transformed closest approach data to fit a normal distribution and ran a linear model.

All statistical tests were conducted in the statistical program R 4.0.3 (packages lme4, nlme, multcomp, emmeans, and car), with $\alpha = 0.05$. For all significant models, we ran post hoc tests with a Tukey correction to compare the least-square means outputs between playback treatment and status pairs.

## 2.4 RESULTS

We conducted a total of 150 playback trials on 89 males that included cowbird chatters (unpaired warbler males n = 11, paired warbler males n = 19), yellow warbler seet calls (unpaired n = 16, paired n = 15), yellow warbler chip calls (unpaired n = 15, paired n = 15), blue jay calls (unpaired n = 13, paired n = 16), and wood thrush song (unpaired n = 12, paired n = 18).

### 2.4.1 Latency

Based on the binomial model, male yellow warblers differed by whether they responded to playbacks depending on the treatment (treatment term: $F_{4, 137} = 3.09$, $p = .01$). Paired males also responded to more playbacks than unpaired males (pairing status term: $F_{1, 137} = 23.02$, $p < .0001$; Figure 8). We did not find a significant interaction between treatment and pairing status (treatment x pairing status term: $F_{4, 137} = 0.83$, $p = .50$), or an effect of date (date term: $F_{1, 137} = 0.87$, $p = .35$) on latency. Based on post hoc pairwise comparisons, paired males were less likely to respond to control wood thrush playbacks compared to cowbird chatters ($z = -3.7$, $p < .01$), blue jay calls ($z = -3.51$, $p < .01$), chip calls ($z = -3.65$, $p < .01$), and seet calls ($z = -3.51$, $p < .01$). All pairwise comparisons within unpaired males were non-significant (see Supplementary Table 3). Paired males also responded to more cowbird chatter ($z = 3.24$, $p < .01$), blue jay ($z = 3.24$, $p < .01$), and seet playbacks ($z = 2.39$, $p = .01$) than unpaired males.

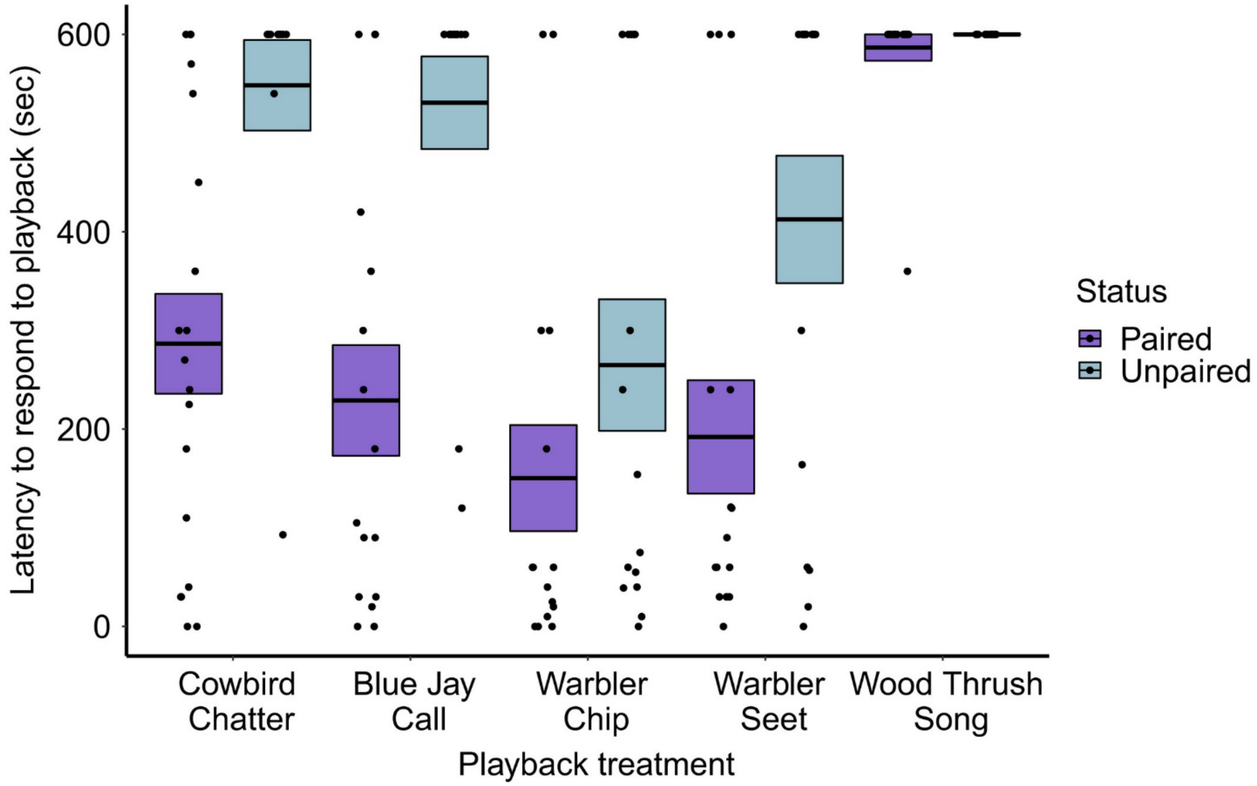

**Figure 8.** Mean latency (sec) of paired and unpaired male yellow warblers to respond to each playback treatment. Means are shown with the bold line, and shaded boxes represent standard errors. The graph includes latency data for non-responders (600s)

When we compared latencies of warblers who responded to the treatment playbacks (the control was dropped for this analysis because only one warbler responded), we found significant differences across treatments (treatment term: F3, 63 = 2.68, p = .05) but not pairing status (pairing status term: F1, 63 = 0.37, p = .54). Specifically, paired warblers responded more quickly to playbacks of cowbird chatter compared to chip calls (z = 2.7, p = .04). All other pairwise comparisons for treatment were non-significant. Additionally, there was no effect of date of playback (date term: F1,63 = 0.68, p = .41), and no interaction between treatment and status (treatment x pairing status term: F3,63 = 0.13, p = .93).

### 2.4.2 Closest approach

Closest approach was significantly influenced by pairing status (pairing status term: F1, 137 = 7.63, p < .01; Figure 9), in that paired birds approached the speaker more closely than unpaired males. Treatment did not significantly influence closest approach (treatment term: F4, 137 = 1.45, p = .21), nor did date (date term: F1, 137 = 0.03, p = .84). There was also no significant interaction between treatment and pairing status on closest approach (treatment x pairing status term: F4, 137 = 0.95, p = .43).

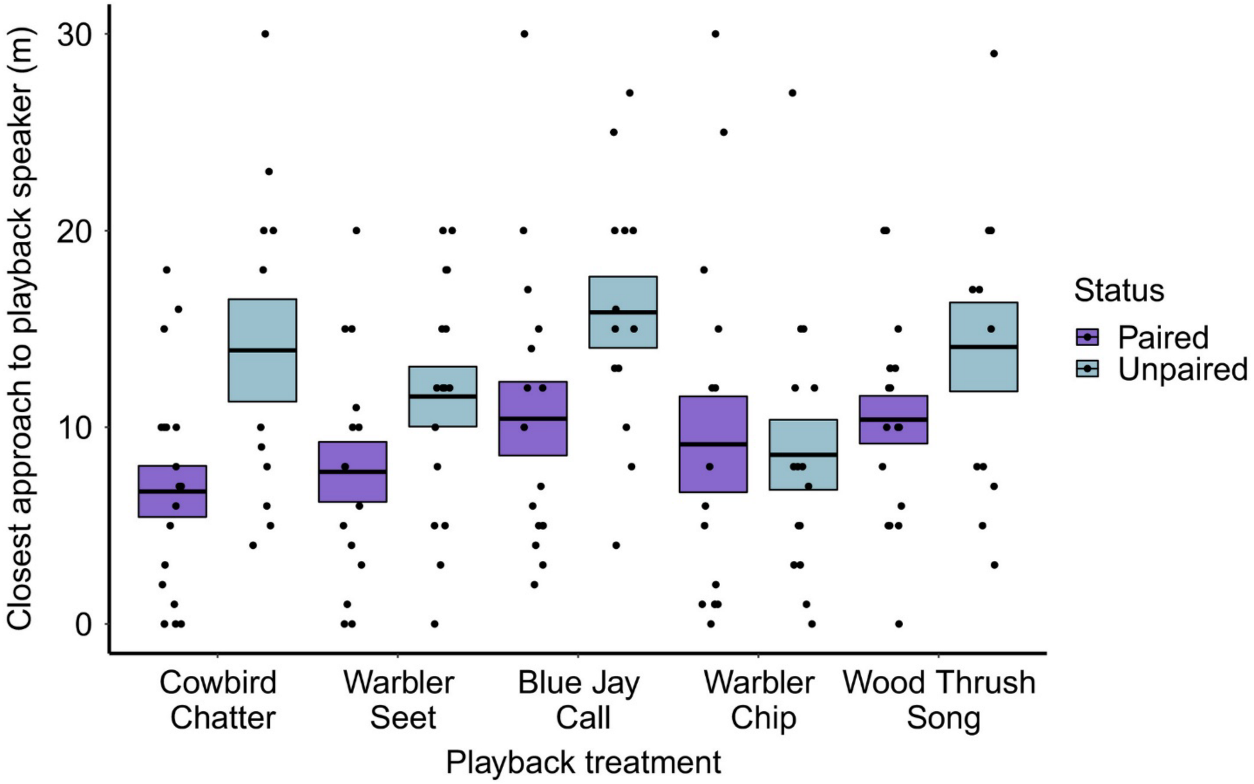

**Figure 9.** Mean closest approach (m) to playback speaker by paired and unpaired male yellow warblers for each playback treatment. Means are shown with the bold line, and shaded boxes represent standard errors

### 2.4.3 Seet call production

Playback treatment (treatment term: F4, 137 = 28.22, p < .001) significantly affected whether birds produced any seet calls or not (Figure 10). Males only ever seet called during cowbird and seet playbacks, so only these playbacks were compared using post hoc pairwise comparisons to every other treatment. We also found that paired males seet called more toward all playbacks than unpaired males (status term: F1, 137 = 33.64, p < .001), with unpaired males only ever producing seet calls during seet playbacks. There was no significant interaction between treatment and pairing status (treatment x pairing status term: F4,137 = 1.78, p = .13), and neither year (year term: F1,137 = 0.03, p = .86), date (date term: F1,137 = 0.01, p = .90) nor trial order (trial term: F1,137 = 3.3, p = .06) had a significant effect on whether seet calls were produced. Paired males were more likely to seet call during cowbird chatter trials compared to blue jay (z = 2.39, p = .04), chip call (z = 2.31, p = .05), and wood thrush trials (z = 2.40, p = .04) (see Supplementary Table 4). Similarly, paired warblers were also more likely to seet call in response to seet trials compared to blue jay (z = 2.53, p = .03), chip call (z = 2.45, p = .03), and wood thrush trials (z = 2.54, p = .02). There was no difference in the number of seet and cowbird trials that elicited seet calls from paired warblers (z = −0.38, p = .92). Conversely, unpaired warblers seeted fewer times across all playbacks (see Supplementary Table 4). For paired males, seet production during cowbird chatter and seet call playbacks was not significantly affected by any of our fixed effects: treatment (treatment term: F1, 14 = 2.9, p = .10), year (year term: F1, 14 = 0.01, p = .89), date (date term: F1, 14 = 0.01, p = .92), or trial order (trial term: F1, 14 = 2.11, p = .16).

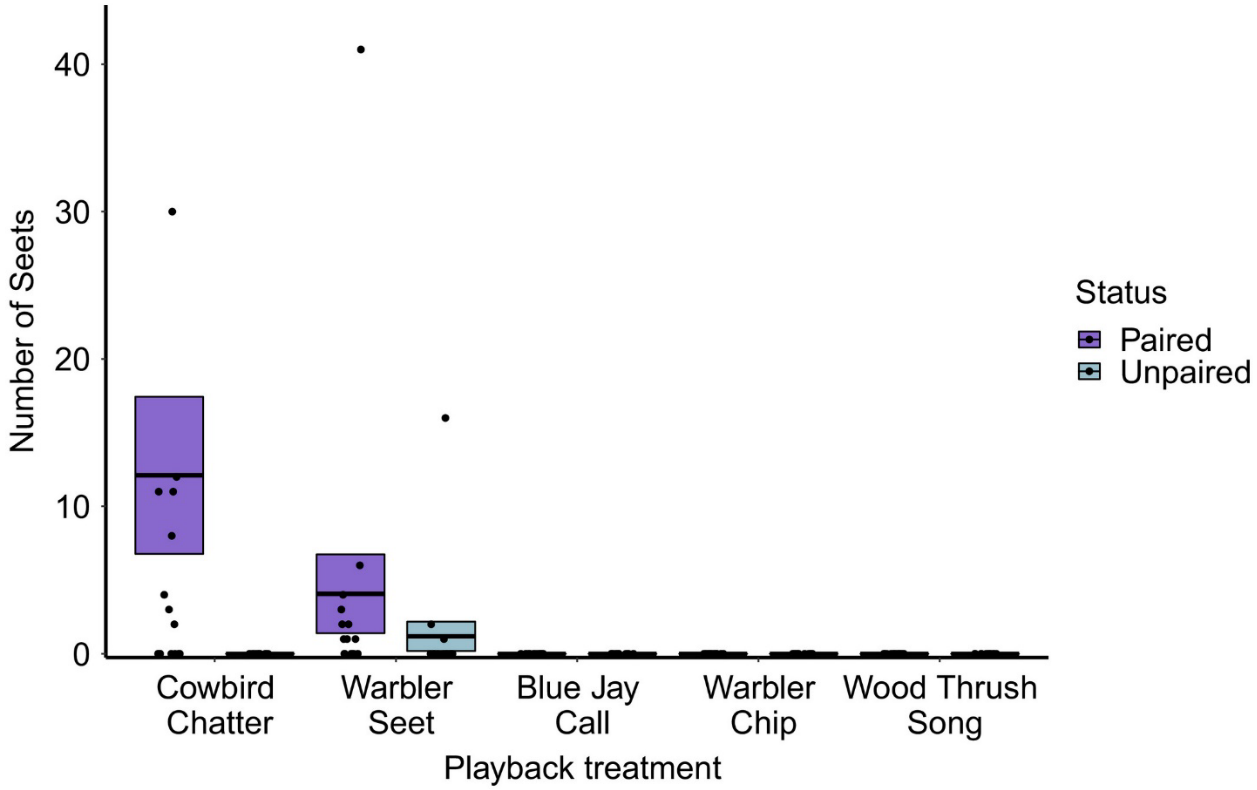

**Figure 10.** Mean number of seet calls produced by paired and unpaired male yellow warblers in response to each playback treatment. Means are shown with the bold line, and shaded boxes represent standard errors. Here, 2 data points with values over 50 are not shown, both for Cowbird Chatter Paired (# seet calls = 72, 77). The graph includes seet data for non-responders (zeros)

### 2.4.4 Chip call production

Chip call production was significantly influenced by playback treatment (treatment term: F4, 137 = 11.88, p < .0001; Figure 11) and pairing status (status term: F1, 137 = 12.16, p < .0001), and furthermore, paired males responded differently than unpaired males to different playback treatments (treatment x pairing status term: F4, 137 = 2.9, p = .02). Paired males chipped less to control wood thrush song compared to blue jay calls (z = −4.55, p < .0001), chip call (z = −4.29, p < .001), seet call (z = −3.21, p = .01), and cowbird chatter playbacks (z = 2.76, p = .04). Paired males also chipped less during cowbird chatter trials compared to blue jay (z = −3.49, p < .01) and chip call playbacks (z = −2.91, p = .02). Unpaired males produced more chip calls during chip playbacks than blue jay (z = 2.88, p = .03), seet call (z = 2.74, p = .04), and wood thrush song trials (z = 3.02, p = .02). Paired males produced more chip calls than unpaired males during blue jay ($z = 4.21$, $p < .0001$) and seet treatments ($z = 2.13$, $p = .03$). All other pairwise comparisons for paired and unpaired males were non-significant (see Supplementary Table 5). Date of playback was found to be significant, although it was unclear whether chip calling was higher earlier versus later in the season as chipping varied across treatments (date term: $F1, 137 = 5.83$, $p = .01$). A linear regression with date of playback and treatment on number as fixed effects revealed a significant interaction between the two on number of chip calls produced (date*treatment term: $F4, 137 = 3.52$, $p < .01$). We found with post hoc comparisons by treatment that males chip called significantly more during Blue Jay playbacks as the breeding season progressed ($t = 2.50$, $p = .01$, Supplementary Figure 1), while chip calling during cowbird treatments significantly decreased over the season ($t = -2.37$, $p = .02$). There was no significant difference in slope of seet ($t = -1.46$, $p = .15$), chip ($t = -1.64$, $p = .10$), or wood thrush ($t = -0.96$, $p = .34$) treatments in chip calls produced over the season.

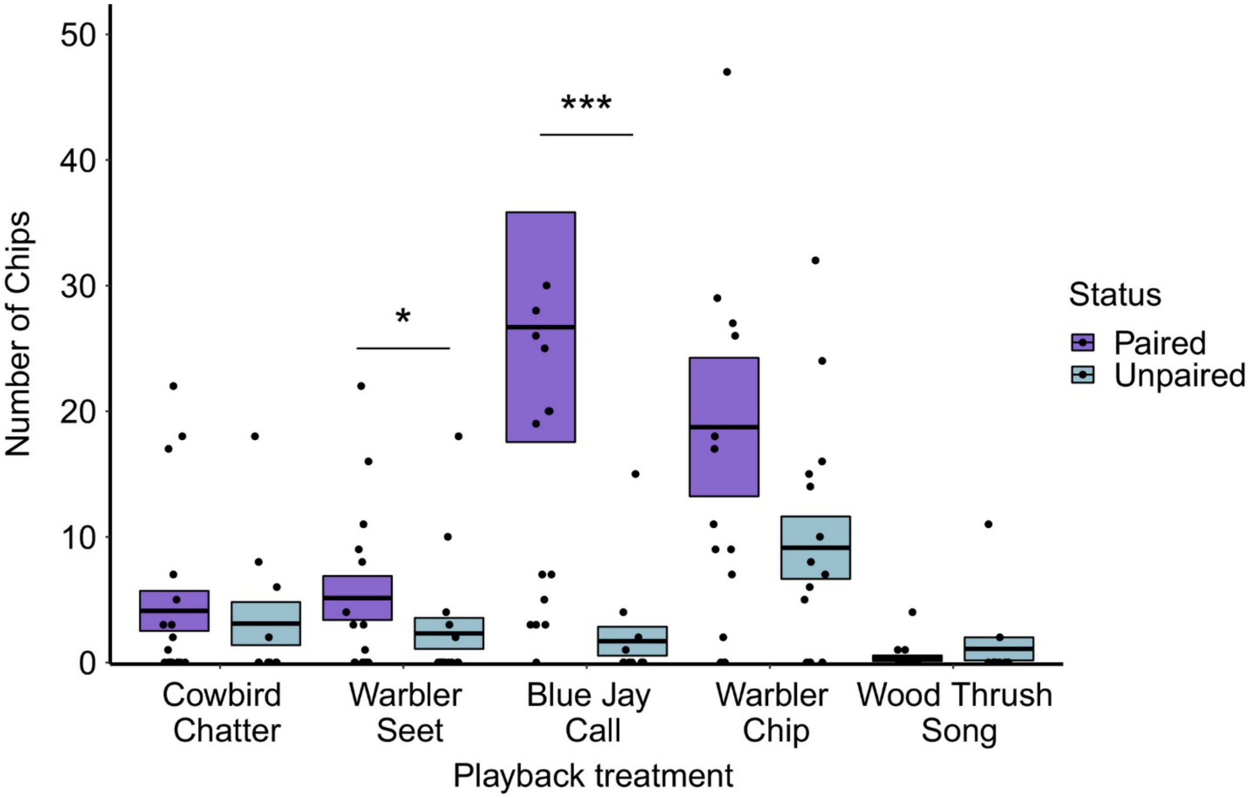

**Figure 11.** Mean number of chip calls produced by paired and unpaired male yellow warblers in response to each playback treatment. Means are shown with the bold line, and shaded boxes represent standard errors. Here, 3 data points with values over 50 are not shown, 2 for Blue Jay Paired (# chips = 131, 100), and 1 for Warbler Chip Paired (# chips = 79). Significant post hoc differences between paired and unpaired males are represented with asterisks (*** p < .001, * p < .05)

## 2.5 DISCUSSION

Animals that defend their offspring against predators and other threats must make repeated decisions to align energetic costs of their defenses with the value and extent of their current reproductive investment. Studies of nest defense in birds show that hosts of avian brood parasites use visual and social cues to discriminate between predatory and brood parasitic threats (Gill & Sealy, 2003; Kuehn et al., 2016; Lawson et al., 2020a; Neudorf & Sealy, 1992; Welbergen & Davies, 2008), and mediate the intensity of their aggressive response depending on nesting stage (specifically, laying/incubation versus nestling stages; Campobello & Sealy, 2010; Duckworth, 1991; Gill & Sealy, 1996; Neudorf &

Sealy, 1992). Our study expands on these earlier findings and demonstrates that male yellow warblers adjust their nest defense intensity before versus after the onset of a breeding attempt.

Male subjects' response levels toward nest threats varied in our experiment depending on current breeding status. Paired males were more likely to respond to and closely approach playbacks of cowbird chatters, seet calls, and blue jay calls than unpaired males. Unpaired males often did not respond at all to these playbacks. Paired males were also more vocal than unpaired males toward all non-control playbacks, producing more chip calls in response to nest-predatory blue jay calls in particular, and more often seet calling in response to cowbird chatter and seets than unpaired males. On the other hand, across three of our measured behaviors (latency, approach, number of seet calls) unpaired male responses were never significantly different between treatment playbacks of nest threats and the control wood thrush song, demonstrating that males without nest investments generally do not respond aggressively to stimuli that only threaten nests. While male warblers do not sit upon the nest in response to seet calls like female warblers (Gill et al., 1997a; Hobson & Sealy, 1989a; Kuehn et al., 2016), male warblers in our study show contextual differences in response to seets depending on pairing status. This builds on past studies that found differences in response depending on nest stage (Gill et al., 2008; Hobson & Sealy, 1989a), and provides further support that the seet call is functionally referent and production of seet calls is dependent on brood parasitism risk.

Interestingly, we found that while unpaired males never seet called at cowbird chatters, a few did seet call during seet playbacks, despite having no nest to defend from brood parasitism. Without a nest to defend, unpaired males would waste time and energy responding to signals indicating the presence of cowbirds. It is possible that because the seet calls used for our study were recorded from female conspecifics (Gill & Sealy, 2003), those unpaired males that responded to these calls were more interested in the simulated female warbler rather than the warning of the referential call itself. Rates of extra-pair copulations (EPCs) are high in yellow warblers (Yezerinac & Weatherhead, 1997a, 1997b) and males that are unsuccessful in gaining a mate may seek out EPCs from paired females that are currently laying eggs, which also is when females would be most likely to produce seet calls. Following our experiment, we did capture and band several warblers from our study and found, based on plumage characteristics (Pyle, 1997), that paired males were almost exclusively older males (second year or later) that would have had experienced at least one prior breeding season (n = 56 of 89 males in this study banded afterward, S.L.L. unpublished data). This begs the question of whether other life-history factors likely related to pairing status, such as age and previous experience with cowbird brood parasitism, play a role in yellow warblers' decision to respond to other seet calls, especially because yellow warblers appear to pay an age-specific cost for cowbird parasitism (Rock et al., 2013). It is also possible that some unpaired males still participate in group defense against cowbirds, by seet calling along with conspecifics as a “neighborhood watch” response to social information revealing brood parasitism risk (e.g., Campobello & Sealy, 2011a; Krams, Berzins, et al., 2010; Krams et al., 2010; but see Campobello & Sealy, 2011b).

Context also influenced the production of chip calls by male yellow warblers. Unlike the pattern seen with seet calls, unpaired males did vary in their chip call production between treatments, specifically in that they were most vocal during chip playbacks compared to the other treatments. This result is important because it demonstrates that unpaired males are responsive to some conspecific signals (chips) but not all (seets), presumably reflecting their salience to them. Chip calls can signal a variety of threats not necessarily related to the nest, such as nearby predators (of adult warblers) and conspecifics in the territory (Gill & Sealy, 2003; Hobson & Sealy, 1989a), both of which are contextually relevant signals even for unpaired males. Moreover, because a variety of stimuli elicit chip calls, warblers may require visual cues to fine tune their responses when they detect these calls. Here, without the benefit of visual cues, unpaired males may have responded to chip calls because they also can signal the presence of female warblers, and a quick response to chip calls could result in pairing. Interestingly, chip production for yellow warblers in general also varied over the breeding season for some treatments, with significantly more chip calls being produced toward blue jay playbacks later in the season, and less chip calls toward cowbirds. Similar patterns have been observed in other alarm-calling species from nest predator (see Montgomerie & Weatherhead, 1988, for review) and brood parasite (Brooke et al., 1998; Trnka et al., 2013) presentation studies. These responses may be due to lower benefits of renesting later in season and the typically reduced parasitism pressure over time (Montgomerie & Weatherhead, 1988; Trnka et al., 2013). Together, our data show that yellow warblers responses are flexible depending on risk to both the male himself and his breeding attempt, and males adjust their aggressive responses to different threat signals accordingly.

Our study is also the first to assess whether acoustic cues alone are sufficient for male yellow warblers to respond to brood parasitism risk. Specifically, we show adult male yellow warblers produce seet calls in response to cowbird chatter playbacks, as Gill et al. (1997a) only reported grouped seet-calling responses of both sexes to chatter playbacks. Other studies have only recorded male seet calls in response to live or model cowbirds (Campobello & Sealy, 2011a; Gill et al., 1997a, 2008; Guigueno & Sealy, 2011). Playbacks present an underused but useful presentation stimuli that could be incorporated into future experimental designs on brood parasite–host systems (Lawson et al., 2020a; Louder et al., 2020). It is, nonetheless, possible that playbacks are not as frequently chosen because they tend to attract live brood parasites into the presentation area. Accordingly, even in our study there were a number of playback trials (~50%) where wild female cowbirds naturally approached the chatter call playbacks in the field, which may possibly influence host behavior. However, female cowbird presence during cowbird playbacks was not found to covary with whether male warblers seet called in response to cowbird playbacks (Fisher's exact test, p = .72), nor how many seets were produced (F1, 21 = 0.03, p = .85). This further supports that cowbird chatters, like cowbird models, are a suitable stimulus to emulate the presence of a female cowbird (and associated threat) in playback studies.

In conclusion, our results suggest that non-breeding males, without active reproductive investment in a nest, respond differently to social information about nest threats compared to paired males with an active reproductive investment. These results further support that paired males use seet calls as a specialized and referential call reserved for brood parasitic cowbirds, whereas chip calls are a generic-danger alarm call used in other contexts of different predators or conspecific territory intrusions. Lastly, our study helps us to better understand when in the nesting cycle males begin to pivot their behavior toward responding to nest specific threats, with this window occurring sometime after establishing a territory but before a nest investment is initiated.

# CHAPTER 3: RESPONSES OF FEMALE YELLOW WARBLERS TO PLAYBACKS SIGNALING BROOD PARASITISM AND PREDATION RISK: A QUASI-REPLICATION STUDY

## 3.1 ABSTRACT

Many avian species are negatively impacted by obligate avian brood parasites, which lay their eggs in the nests of host species. The yellow warbler (*Setophaga petechia*), which is host to the brood-parasitic brown- headed cowbird (*Molothrus ater*), represents one of the best-replicated study systems assessing antiparasitic host defenses. Over 15 prior studies on yellow warblers have used model-presentation experiments, whereby breeding hosts are exposed to models of brown-headed cowbirds or other nest threats, to test for anti-parasitic defenses unique to this species. Here we present results from our own quasi-replication study of the yellow warbler/brown- headed cowbird system, which used a novel design compared to previous experiments by pivoting to conduct acoustic playback treatments only, rather than presenting visual models with or without calls. We exposed active yellow warbler nests to playbacks of brown-headed cowbird chatters (brood parasite), blue jay (*Cyanocitta cristata*; nest predator) calls, conspecific "seet" calls (a referential alarm call for brood parasitism risk), conspecific "chip" calls (a generic alarm call), or control wood thrush (*Hylocichla mustelina*; harmless heterospecific) songs during the incubation stage. Similar to previous studies, we found that female yellow warblers seet called more frequently in response to playbacks of both brood parasitic chatter calls and conspecific seet calls whereas they produced more chip calls in response to the playback of nest predator calls. In contrast, female yellow warblers approached all playbacks to similar distances, which was different from the proximity patterns seen in previous studies. Our study demonstrates the importance of both replicating, and also pivoting, experimental studies on nest

defense behaviors, as differences in experimental design can elicit novel behavioral response patterns in the same species.

## 3.2 INTRODUCTION

The fitness of over 200 North American passerine species is known to be negatively impacted by the brown-headed cowbird (*Molothrus ater*, hereafter "cowbird"), an obligate avian brood parasite that lays its eggs in the nests of other species, leaving the host to care for the parasitic young (Davies, 2010). The behavioral defenses that hosts use to prevent costly cowbird parasitism have been well-studied using similar experimental design paradigms: typically, the host is exposed to stimuli representing this brood parasite and procedural controls, and the host's behavioral responses are compared across treatments (e.g., Briskie et al., 1992; Robertson & Norman, 1976a; Sealy et al., 1998).

The yellow warbler (*Setophaga petechia*), a common host for cowbirds, represents one of the best replicated study systems assessing behavioral defenses related to host-parasite interactions (see Appendix for summary). This is perhaps because yellow warblers have a unique, referential anti-cowbird call, known as the seet call, that they use to warn conspecifics of nearby female parasites (Gill et al., 1997a, b; Gill & Sealy, 2004; Hobson & Sealy, 1989a). The seet call is produced by both males and female warblers in response to cowbirds, and females that hear this call rush back to sit on their nests to presumably prevent cowbirds from inspecting and parasitizing the nest (Gill et al., 1997a; Gill & Sealy, 2004). The use of the seet call is strikingly different from the yellow warbler's "chip" call, a general alarm call used to warn of a range of threats, such as predators and intruding conspecifics (Gill & Sealy, 1996; Hobson et al., 1988). The duality of these two alarm call types and the differing socioecological contexts under which they are produced have led to a plethora of experimental studies in the yellow warbler system, both from the same research groups and across different institutions, each study addressing similar questions regarding the seet call, while simultaneously pivoting to add new details of biological knowledge to the system (see Appendix). As such, the cues for and the function of the referential seet calls in yellow warblers may already represent one of the best replicated behavioral, ecological, and cognitive systems in a wild songbird species, while also providing new opportunities to both replicate and pivot from some of the most robust findings in this system.

Several early studies on yellow warbler antiparasitic nest defenses noted aggressive responses from both sexes towards models (in particular, of adult females) of brown-headed cowbirds presented to host pairs at the nest, given that female cowbirds represent the most immediate brood parasitic threat for foreign-egg laying (Folkers & Lowther, 1985; Robertson & Norman, 1976a, b). This experimental approach was then expanded by studies that tested yellow warblers' responses to female cowbird models during different stages of nesting and found that aggression was stronger during laying and incubation (relative to the nestling stage), when hosts are at the highest risks of being successfully parasitized (Burgham & Picman, 1989; Hobson & Sealy, 1989a). In turn, a series of studies by Gill and Sealy characterized how yellow warblers produced seet calls specifically in response to cowbirds, and that this functionally referent response was produced largely during laying and incubation as seen in previous studies examining shifts in behavioral aggression across the nesting stages (Gill et al., 2008; Gill et al., 1997a, b; Gill & Sealy, 1996, 2003, 2004). Additional replication and pivoting studies were conducted that added comparisons between different nest threat types of models (brood parasite versus nest predator; Burgham & Picman, 1989; Campobello & Sealy, 2011a; Gill et al., 1997a; Gill & Sealy, 1996, 2004; Guigueno & Sealy, 2011; Kuehn et al., 2016), stimulus sensory modality (visual model and acoustic playback; Campobello & Sealy, 2011b;

Gill et al., 1997a; Gill & Sealy, 2004; Kelly et al., 2019), and geographic overlap with cowbirds (sympatry versus allopatry; Briskie et al., 1992; Gill & Sealy, 2004; Kuehn et al., 2016) (see Appendix). Taken together, these studies support that seet calls are specifically associated with the presence of the female brood parasite and mainly produced when cowbirds pose an active threat to the nest, whereas chips are produced in response to other types of threats.

Here we conducted our own quasi-replication study in the yellow warbler/brown-headed cowbird system using acoustic presentations as our sole experimental stimuli. We present a novel, playback-only based experimental design to probe anti-parasitic responses of female (this study) and male yellow warblers (Lawson et al., 2021a), as well as heterospecific red-winged blackbirds (*Agelaius phoeniceus*, Lawson et al., 2020a). Accordingly, during laying/incubating stages at yellow warbler nests, we presented playbacks of female cowbird chatters, conspecific seet calls, conspecific chip calls, nest predator calls (blue jay, *Cyanocitta cristata*), as well as procedural controls of songs of a harmless sympatric heterospecific, the wood thrush (*Hylocichla mustelina*). We then compared behavioral responses of female yellow warblers across treatments. There is also novelty in our quasi-replication in that we applied acoustic stimuli alone for the full series of playbacks. We predicted that female warblers would 1) produce seet calls exclusively in response to cowbird chatter and seet call playbacks, 2) produce more chip calls in response to blue jay calls compared to other playbacks, and 3) respond more quickly and approach all playbacks more closely than the control wood thrush playback.

## 3.3 METHODS

### 3.3.1 Sites and study species

The experiment was conducted at multiple wetland sites in Champaign (n = 3), Iroquois (n = 1), and Vermillion counties (n = 3) in central Illinois, USA, where yellow warblers commonly serve as hosts to cowbird parasitism (Kelly et al., 2019; Lawson et al., 2020a; Merrill et al., 2017). Sites were comprised of mesic shrubland habitat, with dominant shrubs including willow (Salix spp.), dogwood (Cornus spp.), and autumn olive (Elaeagnus umbellate) (Kelly, 2017; Kelly et al., 2018; Lawson et al., 2020a). Yellow warblers are neotropical migrants that arrive at our sites in late-April and breed from early-May through late-June, with a peak and synchronous period of breeding during mid-to-late-May (Kelly et al., 2019, Lawson et al., 2020a).

These studies were approved by the animal ethics committee (IACUC) of the University of Illinois (#17259), and by USA federal (MB08861A-3) and Illinois state agencies (NH19.6279).

### 3.3.2 Playback stimulus construction

Playback files and methodology for this experiment follow those from another playback experiment conducted in the same yellow warbler population (Lawson et al., 2020a). In short, we created playlists for five different playback treatments: (1) female cowbird chatters, (2) conspecific seet calls, (3) conspecific chip calls, (4) blue jay calls, and (5) wood thrush songs.

Audio files were obtained from Xeno-Canto (Blackburn et al., 2014), all sourced from the Midwestern United States (Illinois, Michigan, Minnesota, and Ohio), except for seet calls, which were sourced from Manitoba, Canada, by Gill and Sealy (2003). Playlists were created using Adobe Audition CC 2018. To minimize signal-to-noise ratio, frequencies below 500 Hz, which are well lower than the range of any of our call stimuli, were filtered out. Vocalizations from at least three different individuals comprised each playlist exemplar in a random order and then repeated to create the 10-minute playlist. To further reduce pseudoreplication (Kroodsma et al., 2001), we constructed five different files per playback type, and chose one exemplar file randomly for each trial (described below). Intervals of silence were inserted between vocalizations, ranging from two to six seconds based on rates found in natural recordings on Xeno-Canto. Playbacks were broadcast at ~90 dB SPL (measured 0.5 m from speaker).

Playbacks were presented from an AYL-SoundFit speaker connected to a Samsung Galaxy 8 cellular phone loaded with the audio files. We placed the speaker ~1 m high in vegetation and recorded data from > 10 m away. Each playback trial lasted for 10 min.

### 3.3.3 Determining the nesting status of females hosts

We conducted playback trials on active yellow warbler territories with sighted females between mid-May and late-June in 2018 and 2019. We first systematically visited each site twice weekly, two to three days apart, to search for actively singing males with a female on the territory. During the visits we followed each male at the site for 10 min and noted any mate-guarding. Mate-guarding is a behavior commonly used to assign pairing status in parulid (wood) warblers, where males closely follow their mate while she is laying (yellow warblers: Hobson & Sealy 1989b; other parulid warblers: Chuang-Dobbs et al., 2001; Stutchbury et al., 1994; Toms, 2012). We also searched the territories (within ~30 m of male's song perch; Kelly et al. 2018) for active nests and/or females exhibiting nest defense behaviors (e.g., alarm-calling, perch-switching, wing-flicking, circle-flight), which have been used to assign pairing status in similar studies (Ficken & Ficken, 1965; Hobson & Sealy, 1989b; Marshall & Balda, 1974; Mitra, 1999).

We tested a female if we observed mate-guarding by the male and/or evidence of an active nest at least two visits in a row. Furthermore, females were assessed only if their nest was in the laying or incubation stage, when these warblers are most likely to give seet calls (Gill et al., 2008, Gill &

Sealy, 1996). We verified nest stage prior to trials by checking on known nests to confirm their content, or if the nest location was unknown, by observing females for signs of incubation/laying but not of the other nesting stages (e.g., carrying nesting material, carrying food for nestlings or fledglings, etc.).

### 3.3.4 Playback trials

Playbacks occurred between 0500 and 1200 hr local time. Because we did not band territorial birds for individual identification, we tested nests that were ≥ 30 m apart to attain biological independence. Nests at this distance likely belonged to different breeding units based on average territory size of yellow warblers (DellaSala, 1986; Kendeigh, 1941), including those breeding in Illinois (Kelly et al., 2018). We also waited 30–60 min between playbacks at neighboring sites to avoid any carryover effects on individuals in the proximity (Lawson et al., 2020a).

Within the mostly agricultural landscape of Central Illinois, the number of breeding female yellow warblers at our study sites was limited and so, to increase sample sizes, female subjects were tested again with a second, different playback type. We allowed 24–72 hrs (mean = 47.1) between trials to avoid habituation, and used another, randomly assigned treatment to minimize order effects. Nonetheless, we still included order as a fixed predictor in our statistical models (see below).

If a nest location was known, the speaker was placed 5–6 m from the nest; otherwise, the playback speaker was placed 5–6 m from the male's most commonly used song post. During the playback trials, we recorded the female's behavioral responses, as follows, within 30 m of the speaker: (1) response latency (seconds after the start of trial when a switch to aggressive behaviors occurred: posturing, hopping, alarm calling, or attacking the speaker); (2) number of seets; (3) number of chips; and (4) closest approach to the speaker (meters). On territories where nest location was known, we also recorded whether females returned to sit on and protect their nests after hearing or producing seet calls. Thirteen females/sites could not be retested with a second playback (n = 6 in 2018, n = 7 in 2019) either because their nests were depredated between trials or because they were not present for the second trial.

### 3.3.5 Statistical analyses

We evaluated whether playback treatment affected the four behavioral response variables collected (latency, number of seets, number of chips, and closest approach) using separate general linear models for each. All models included playback treatment, year (2018 or 2019), date (ordinal days after start of season - May 1st), and trial order (first or second, to account for repeated playbacks at the same site) as fixed effects. For three of the variables (latency, number of seets and number of chips), there were a large number of biologically relevant non-responses (i.e., subject never responded or produced no vocalizations). As such, these variables were first analyzed using zero-inflated negative binomial models, which analyze the data as a binomial variable (response versus no response) to determine whether non- responses were more common depending on the treatment. We then separately analyzed only data where females had responded (< 600 seconds latency, seet called, and/or chip called) to determine whether the magnitude of the responses (seconds of latency or the number of seet/chip calls) varied between treatments. For this second set of models (responses only) we analyzed the latency and seet data using a general linear model with a Poisson fit and log-e (ln) transformed the chip data to fit a normal distribution and ran a linear model. Lastly, we log-e (ln) transformed the closest approach data to fit a normal distribution and ran a linear model.

We also evaluated whether females with known nest locations were more likely to return to the nest quickly after hearing or producing seet calls, using two separate general linear models with a binomial fit; the first one to test female return within one minute of playback, and the second one to test female return within 3 min.

All statistical tests were conducted in the statistical program R 3.5.2 (packages lme4, nlme, multcomp, emmeans and car), with α = .05. For all significant models we ran post hoc tests with a Tukey correction to compare the least-square means outputs between playback treatments and provided z-scores and 95% confidence intervals as measures of effect size.

## 3.4 RESULTS

We conducted 84 total playback trials at n = 49 nest locations, that included cowbird chatters (n = 20), Yellow warbler seet calls (n = 16), Yellow warbler chip calls (n = 15), Blue jay calls (n = 13), and Wood thrush songs (n = 20).

### 3.4.1 Response and its latency

Based on the binomial model, female yellow warblers differed in whether they responded to playbacks depending on treatment (treatment term: F(4, 83) = 7.27, p < .001; Figure 12). There was no significant effect of year (year term: F(1, 83) = 1.39, p = .24; estimate = 2.85, 95% CI [0.6, 16.2]), date (date term: F(1, 83) = 0.51, p = .47; estimate = 1.02, 95% CI [0.9, 1.1]), or trial order (trial term: F(1, 83) = 0.89, p = .34; estimate = 2.2, 95% CI [0.5, 12.4]) on whether females responded to the playbacks. Based on post hoc pairwise comparisons, female warblers were less likely to respond to control wood thrush playbacks compared to cowbird chatters (z = -3.29, p = .008), blue jay calls (z = -2.71, p = .04), chips (z = -2.84, p = .03), and seets (z = -3.09, p = .01). All pairwise comparisons between non-control treatments were not significant (Table 2). When we compared latencies of warblers who responded to the treatment playbacks (latency < 600 s), we found no significant differences in how long warblers took to respond across treatments (treatment term: F(4, 65) = 2.37, p = .062), year (year term: F(1, 65) = .04, p = .84; estimate = 0.93, 95% CI [0.8, 0.9]), date (date term: F(1, 65) = 1.29, p = .259; estimate = 1.01, 95% CI [1.0, 1.0]), or trial (trial term: F(1, 65) = 1.79, p = .185; estimate = 1.6, 95% CI [1.5, 1.7]).

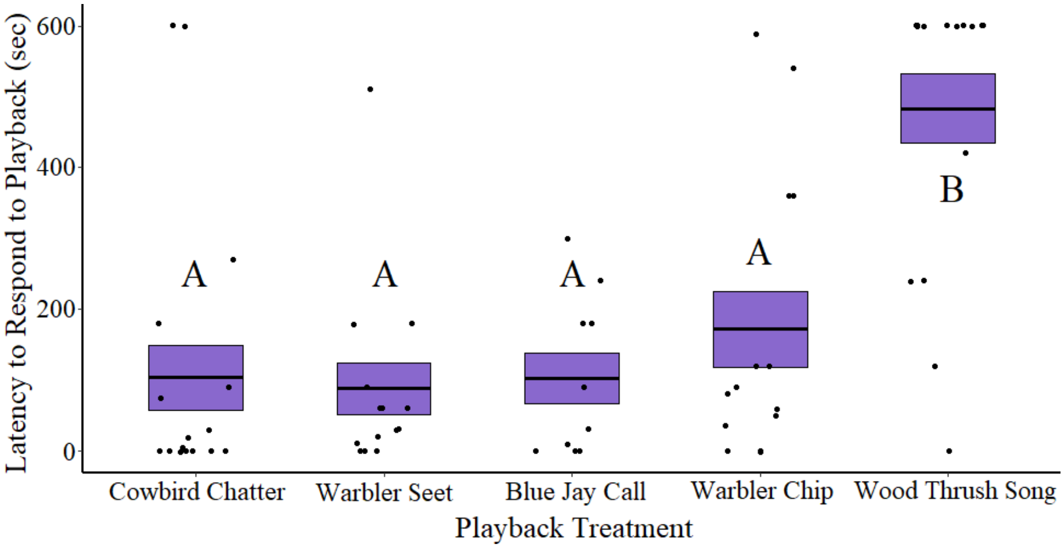

**Figure 12.** Latency of female yellow warblers to respond to each playback treatment. Means are shown with the bold line, and shaded boxes represent standard errors. Boxes with different letters denote post hoc statistical differences between treatments in the binomial model.

**Table 2.** Pair-wise comparisons of latency data by treatment. Brown-headed cowbird chatters (BHCO), yellow warbler seets (Seet), yellow warbler chips (Chip), blue jay calls (BLJA), and wood thrush song (WOTH). Significant post hoc differences are represented with asterisks (*** p < .001, ** p < .01, * p < .05).

| Comparisons (binomial model of response vs no response) | *z* ratio | estimate | CI | *p* value |
|---|---|---|---|---|
| BLJA – BHCO | -0.12 | -0.13 | -2.09 – 1.83 | .999 |
| Chip – BHCO | 0.91 | 1.43 | -0.53 – 3.39 | .886 |
| Seet – BHCO | 0.29 | 0.31 | -1.65 – 2.27 | .998 |
| WOTH – BHCO | -3.29 | -2.88 | 4.85 – (-.92) | .008** |
| Chip – BLJA | 0.94 | 1.57 | -0.39 – 3.53 | .875 |
| Seet – BLJA | 0.36 | 0.45 | -1.51 – 2.41 | .995 |
| WOTH – BLJA | -2.71 | -2.75 | -4.71 – (-0.79) | .048* |
| Seet – Chip | -0.67 | -1.11 | -3.07 – 0.85 | .960 |
| WOTH – Chip | -2.84 | -4.32 | -6.28 – (-2.36) | .033* |
| WOTH – Seet | -3.09 | -3.20 | -5.16 – (-1.24) | .015* |

### 3.4.2 Seet call production

Playback treatment (treatment term: F(4, 83) = 27.16, p < .001) significantly affected whether female warblers produced any seet calls or not (Figure 13). Neither year (year term: F(1, 83) = 3.26, p = .07; estimate = 2.60, 95% CI [0.5, 13.4]), date (date term: F(1, 83) = 1.21, p = .27; estimate = 0.97, 95% CI [0.8, 1.0]), nor trial order (trial term: F(1, 83) = 1.81, p = .18; estimate = 0.49, 95% CI [0.1, 2.1]) had a significant effect on whether females produced any seets. Yellow warblers only seet called during cowbird chatter and seet call playbacks. As such, only these playbacks were compared using post hoc pairwise comparisons to every other treatment. Female warblers were more likely to seet during cowbird chatter trials compared to blue jay (z = 2.63, p = .02), chip (z = 2.70, p = .01), and wood thrush trials (z = 2.89, p = .01) (Table 3). Similarly, female warblers were also more likely to seet in response to seet call playbacks compared to blue jay (z = 2.50, p = .03), chip call (z = 2.55, p = .02), and wood thrush trials (z = 2.72, p = .01). Female warblers were just as likely to produce seets in response to cowbird chatter as seet call playbacks (z = 0.31, p = .94). Similarly, when we then compared seet call rates between cowbird chatter and seet call playbacks, we found that female warblers gave seets at equal rates during cowbird chatter playbacks compared to seet playbacks (treatment term: F(1, 21) = 1.55, p = .22). Neither year (year term: F(1, 21) = .95, p = .34; estimate = 0.70, 95% CI [0.3, 1.4]), date (date term: F(1, 21) = 1.43, p = .24; estimate = 0.97, 95% CI [0.9, 1.0]), or trial (trial term: F(1, 21) = 1.42, p = .24; estimate = 1.54, 95% CI [0.7, 3.2]) significantly affected seet rate.

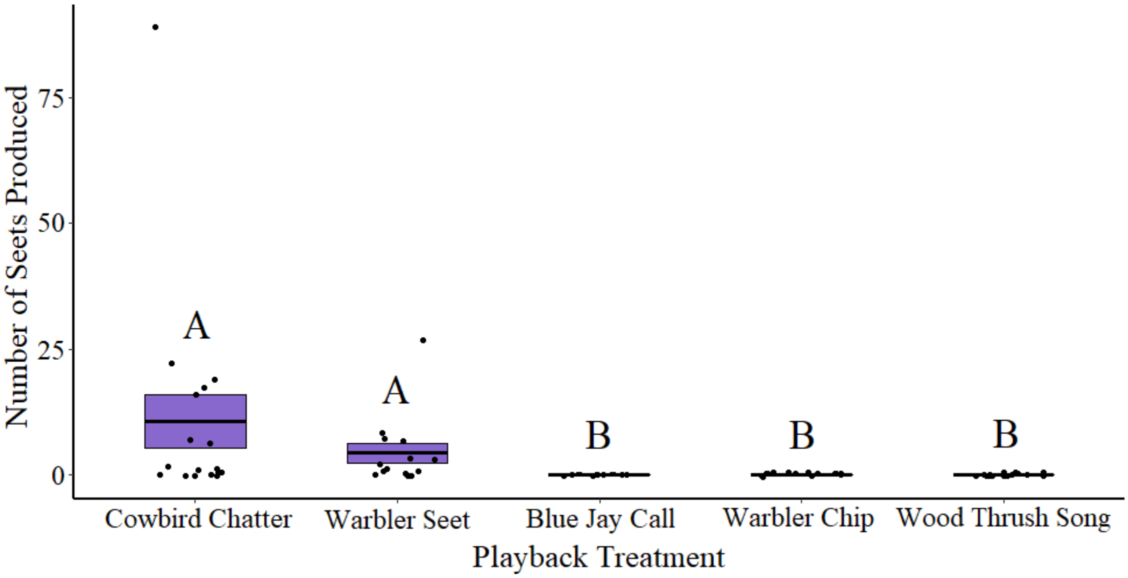

**Figure 13.** Mean number of seets produced by female yellow warblers in response to each playback treatment. Means are shown with the bold line, and shaded boxes represent standard errors. Boxes with different letters denote post hoc statistical differences between treatments in the binomial model.

**Table 3.** Pair-wise comparisons of seet calling data by treatment. Brown-headed cowbird chatters (BHCO), yellow warbler seets (Seet), yellow warbler chips (Chip), blue jay calls (BLJA), and wood thrush song (WOTH). Significant post hoc differences are represented with asterisks (*** p < .001, ** p < .01, * p < .05)

| Comparisons (binomial model of seet vs no seet) | *z* ratio | estimate | CI | *p* value |
|---|---|---|---|---|
| BHCO – Seet | 0.31 | 0.22 | -1.74 – 2.18 | .946 |
| BHCO – BLJA | 2.63 | 4.16 | 2.14 – 6.06 | .022* |
| Seet – BLJA | 2.50 | 3.93 | 1.94 – 5.86 | .033* |
| BHCO – Chip | 2.70 | 4.17 | 2.14 – 6.06 | .019* |
| Seet – Chip | 2.55 | 3.94 | 1.94 – 5.86 | .029* |
| BHCO – WOTH | 2.89 | 4.28 | 2.24 – 6.16 | .010* |
| Seet – WOTH | 2.72 | 4.05 | 2.04 – 5.96 | .017* |

### 3.4.3 Chip production

Playback treatment (treatment term: F(4, 83) = 4.54, p = .002) significantly influenced whether female yellow warblers chipped at all in response to the playbacks (Figure 14). Year of playback was also significant, in that warblers chipped during more trials in 2019 than 2018 (year term: F(1, 83) = 4.60, p = .03; estimate = 3.51, 95% CI [1.1, 11.5]). Neither playback date nor trial order significantly influenced chip rate (date term: F(1, 83) = 2.56, p = .11; estimate = 0.95, 95% CI [0.8, 1.0]; trial term: F(1, 83) = 0.57, p = .44; estimate = 1.55, 95% CI [0.5, 4.7]). With post hoc comparisons we found that blue jay (z = 2.79, p = .03) and chip call (z = 3.15, p = .01) playbacks were more likely to elicit chips from female warblers than the control wood thrush. All other pairwise comparisons between treatments were non- significant (Table 4). We then compared chip call rates of birds who produced any chips during the trial, and found no significant differences between treatments (treatment term: F(1, 83) = 2.25, p = .07), year (year term: F(1, 83) = 0.14, p = .70; estimate = 0.15, 95% CI [-0.6, 0.9]), date (date term: F(1, 83) = 1.37, p = .24; estimate = -0.02, 95% CI [-0.06, 0.01]), or trial (trial term: F(1, 83) = 2.74, p = .10; estimate = -0.64, 95% CI [-1.4, 0.1]).

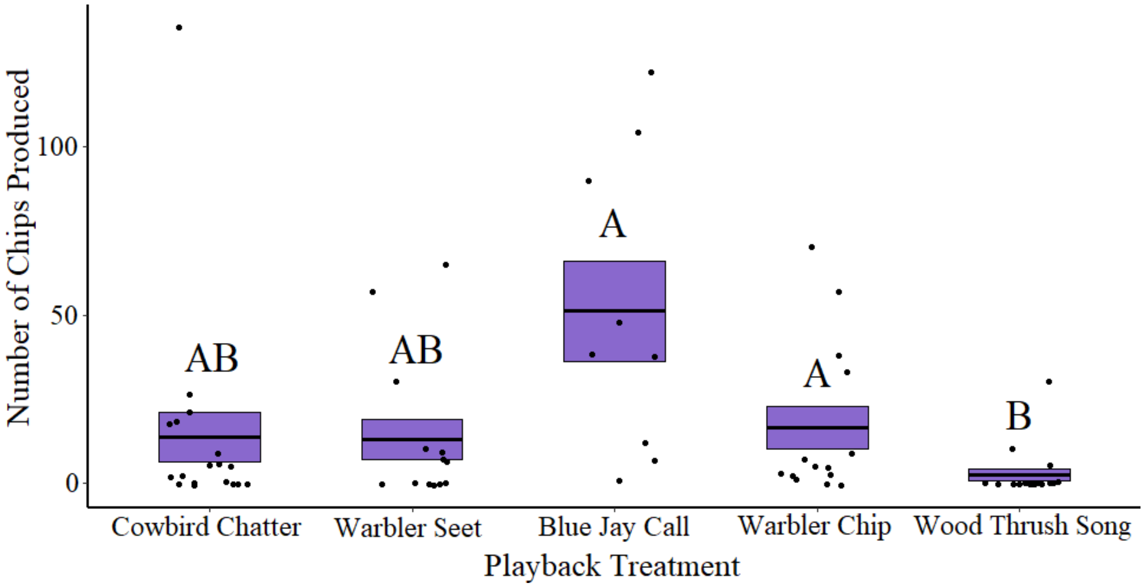

**Figure 14.** Mean number of chips produced by female yellow warblers in response to each playback treatment. Means are shown with the bold line, and shaded boxes represent standard errors. Boxes with different letters denote post hoc statistical differences between treatments in the binomial model.

**Table 4.** Pair-wise comparisons of chip call data by treatment. Brown-headed cowbird chatters (BHCO), yellow warbler seets (Seet), yellow warbler chips (Chip), blue jay calls (BLJA), and wood thrush song (WOTH). Significant post hoc differences are represented with asterisks (*** p < .001, ** p < .01, * p <.05)

| Comparisons (binomial model of chip vs no chip) | *z* ratio | estimate | CI | *p* value |
|---|---|---|---|---|
| BLJA – BHCO | 1.51 | 1.77 | -0.19 – 3.73 | .545 |
| Chip - BHCO | 1.65 | 1.54 | -0.42 – 3.50 | .453 |
| Seet – BHCO | -0.77 | -0.57 | -2.53 – 1.39 | .935 |
| WOTH - BHCO | -2.09 | -1.55 | -3.51 – 0.41 | .214 |
| Chip - BLJA | -0.17 | -0.23 | -2.19 – 1.73 | .999 |
| Seet - BLJA | -1.94 | -2.35 | -4.31 – 0.39 | .284 |
| WOTH – BLJA | -2.79 | -3.33 | -5.29 – 1.37 | .039* |
| Seet – Chip | -2.14 | -2.11 | -4.07 – 0.15 | .192 |
| WOTH – Chip | -3.15 | -3.09 | -5.05 – 1.13 | .013* |
| WOTH - Seet | -1.25 | -0.98 | -2.94 – 0.98 | .712 |

### 3.4.4 Closest approach

Closest approach was not significantly affected by treatment (treatment term: F(4, 83) = 2.15, p =.08), year (year term: F(1, 83) < 0.01, p = .92; estimate = -0.01, 95% CI [-0.3, 0.3]), date (date term: F(1, 83) = 0.03, p = .86; estimate = 0.001, 95% CI [-0.01, 0.02]), or trial order (trial term: F(1, 83) = 1.03, p = .31; estimate = 1.8, 95% CI [-0.1, 0.5]) (Figure 15).

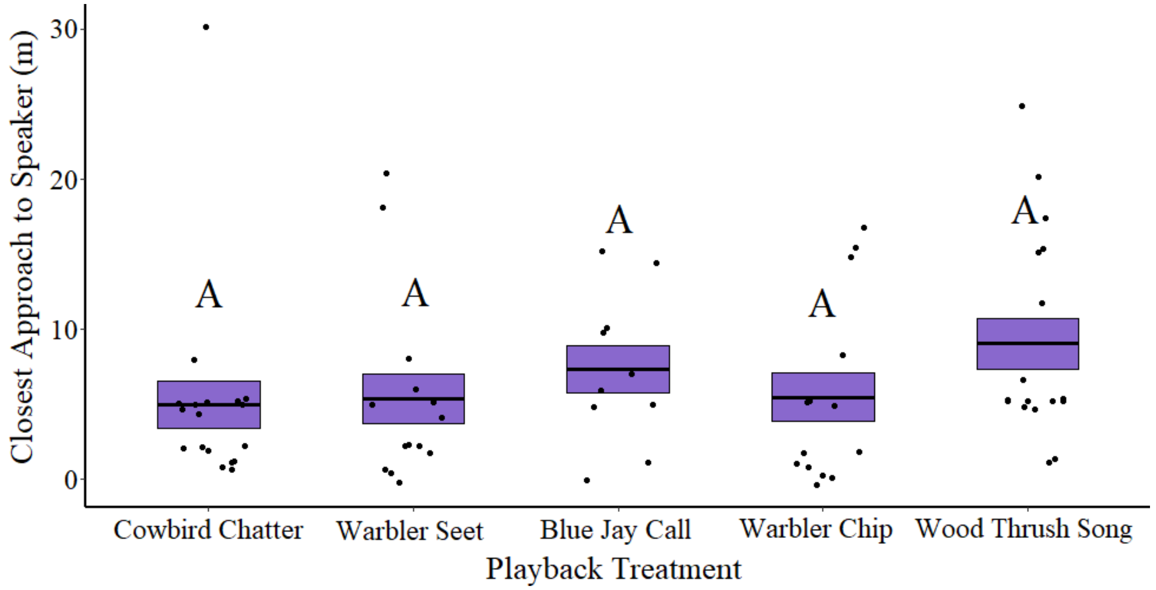

**Figure 15.** Mean closest approach to speaker by female yellow warblers for each playback treatment. Means are shown with the bold line, and shaded boxes represent standard errors. Boxes with the same letter denote no significant post hoc statistical differences between treatments.

### 3.4.5 Nest protection behavior

The majority of female warblers (90%) with known nest locations returned to their nest sometime during the trial after hearing a seet call from the playback or their partner, or after producing a seet call themselves. We found that hearing or producing a seet significantly increased the probability of whether females returned quickly to the nest both within one minute of playback onset (F(1, 30) = 11.27, $p < .01$; 40% returned), and within 3 min (F(1, 30) = 6.28, $p = .017$; 80% returned).

## 3.5 DISCUSSION

Numerous published replication and pivoting experimental studies in the yellow warbler/brown-headed cowbird system have led to an integrated understanding of the seet call as a referential alarm call used in antiparasitic nest defense and the socio-environmental contexts that promote its use (see Appendix). Together with our recent works (Lawson et al., 2020a, 2021a), our study here is amongst the first to use solely acoustic stimuli to compare female yellow warblers' aggressive responses towards a full series of brood parasite, nest predator, and conspecific alarm vocalizations. Indeed, previous studies in this system used mostly either combinations of acoustic/visual stimuli or only visual stimuli. Results from our quasi-replication are highly consistent with those of past model presentation studies: female yellow warblers produced seet calls specifically in response to brood parasitic threats (both cowbird chatter and other seet calls), whereas chip calls were produced more generally in response to nest predators and intruding conspecifics. In turn, as reported before (see Appendix), upon hearing seets produced by playbacks, their mate, or themselves, female warblers predictably returned to sit upon their active nest with eggs.

Many of our results were similar to those of previous studies in this system, in that female host aggression responses varied depending on the nest threat at hand. Females responded swiftly to all experimental treatments relative to the control playback, though there was no difference in latency between experimental treatments. Female hosts produced seet calls only during cowbird chatter and conspecific seet call playbacks, adding further support to the specificity of the seet call and its use as a referential warning for a nearby brood parasitic threat (Evans et al., 1993; Gill & Bierema, 2013). Another similarity to previous work was that a majority of female yellow warblers rushed back to and sat on their nests swiftly after hearing or producing seet calls, a key behavioral response reported in previous studies that defines the responses to seet calls as an adaptive, anti-parasitic nest defense behavior (Gill & Bierema, 2013; Gill & Sealy, 2004). We found that female hosts produced more chip calls during blue jay and chip call playbacks compared to all other treatment and control playbacks. Previous studies have characterized chips as calls produced primarily in response to general threats (predators of nests and/or nesting adults) and towards conspecifics that invade a territory (Gill & Sealy, 1996: Hobson et al., 1988; Hobson & Sealy, 1989b). In experimental studies that specifically compared responses to a cowbird versus a nest predator model, the nest predator model elicited more chip calls (Gill & Sealy 1996, 2004; Kuehn et al., 2016), similar to the qualitative, but not statistically different, patterns seen in our study.

Our quasi-replication found some differences from previous studies, as well. Latency to respond had been used as a response metric only once before in Gill et al. (1997a) who found that yellow warblers responded more quickly to female cowbird models compared to male or control models. In our study, females responded more swiftly to playbacks of cowbird chatters, seet calls, chip calls, and blue jay calls compared to control wood thrush songs, though there was no statistical difference in latency between the non-control treatments. For the response metric of closest approach, we found no statistical difference between experimental treatments, in that female yellow warblers approached all playbacks similarly close. This is distinct from the findings of Gill and Sealy (1996) who found that females approached the experimental models more closely than the control, and in particular, the cowbird model more closely than the nest predator common grackle (*Quiscalus quiscula*) model (also see Gill et al., 1997a for similar results with morphologically modified cowbird models). The use of acoustic playback presentation alone generates no visual target for responding subjects to home in on, which may result in different findings for closest approach compared to model presentation studies. In our study, there were a number of chatter call playback trials (~50%) where live female cowbirds naturally approached the playback, possibly influencing the female yellow warblers' seet calling and nest return responses. However, cowbird presence during cowbird playbacks did not statistically covary with whether warblers seet called in response to the chatter playback (Fisher's exact test, p = .37), indicating that cowbird chatters, like cowbird models, are a suitable stimulus to experimentally simulate cowbirds. Female warblers seet called equally during cowbird chatter playbacks than during seet playbacks, but in our playback study with male yellow warblers (Lawson et al., 2021a), we found that males seet called at a higher rate towards cowbird chatters compared to seet call playbacks. Personal (private) information (sensu Thorogood & Davies, 2012) about brood parasitism risk, such as directly seeing or hearing the brood parasite, can offer greater reliability of the threat compared to social information obtained from assessing cowbird presence based on seet calls from neighbors. Additionally, the role of personal versus social information has also been recorded in other alarm-calling species in response to predation risk (e.g., Carlson et al., 2020). Stronger responses to social information of the seet call by female yellow warblers in comparison to males may be because females primarily stay hidden when incubating the nest, and thus may be more dependent on information about cowbird risk from others.

Future studies could expand on whether stimulus modality (visual vs. acoustic) affects the magnitude of alarm calling and other behavioral responses towards the threat, and if this varies with nest stage or sex of the target subject. Studies could also be conducted to compare responses of experienced (2+ years old) and inexperienced breeder (1 year old) yellow warbler females to various playbacks, as first year yellow warblers of both sexes can be reliably aged in the spring (Pyle, 1997). Hobson and Sealy (1989a) presented cowbird models to female yellow warblers of varying ages and found that older warblers responded more aggressively to the model, which suggests that long-term prior personal experience plays a role in response to brood parasitic cowbirds.

Our results demonstrate the importance of both replication and refinement of methodology in stimulus presentation studies meant to elicit specific behaviors, including alarm calls. The referential function of the yellow warbler's seet call and the specific contexts in which it is produced has become known through a series of replication and pivoting studies aimed at an increasingly better understanding of its use as an anti-parasitic alarm call. Our study further supports the yellow warbler's seet calls' role as a functionally referent signal to alert about brood parasitism risks near host nests (see Appendix; also see Lawson et al., 2020a for its use in heterospecific communication systems), whereas the chip call serves as a generic anti-predatory alarm call. We conclude anew that acoustic stimuli of both cowbirds and nest predators are sufficient to elicit yellow warbler seet and chip calls, respectively, but the magnitude of some behavioral responses may vary depending on the sensory modality of the presentation stimulus used.

# CHAPTER 4: REFERENTIAL ALARM CALLING ELICITS FUTURE VIGILANCE IN A HOST OF AN AVIAN BROOD PARASITE

## 4.1 ABSTRACT

Yellow warblers (*Setophaga petechia*) use referential 'seet' calls to warn mates of brood parasitic brown-headed cowbirds (*Molothrus ater*). In response to seet calls during the day, female warblers swiftly move to sit tightly on their nests, which may prevent parasitism by physically blocking female cowbirds from inspecting and laying in the nest. However, cowbirds lay their eggs just prior to sunrise, not during daytime. We experimentally tested whether female warblers, warned by seet calls on one day, extend their anti-parasitic responses into the future by engaging in vigilance at sunrise on the next day, when parasitism may occur. As predicted, daytime seet call playbacks caused female warblers to leave their nests less often on the following morning, relative to playbacks of both their generic anti-predator calls and silent controls. Thus, referential calls do not only convey the identity or the type of threat at present but also elicit vigilance in the future to provide protection from threats during periods of heightened vulnerability.

## 4.2 INTRODUCTION

Diverse lineages of mammals and birds exhibit referential communication: signals refer to specific objects in the environment, and others receiving such signals respond accordingly, revealing that these listeners understand what is being signalled (Gill & Bierema, 2013; Suzuki, 2016). For example, referential alarm calls in most species studied alert conspecifics of specific predatory threats, such as aerial-hunting versus ground approaching predators, and the signal alone is sufficient to elicit escape responses by conspecifics appropriate for the type of predator (Seyfarth et al., 1980). More rarely, referential alarm calls specifically denote the presence of a very different threat: obligate brood parasitic birds that lay their eggs in the nests of other species (Gill & Bierema, 2013). When hosts detect anti-parasitic referential calls, they immediately respond with mobbing (Feeney et al., 2013) or by physically protecting the nest from the parasite (Gill & Sealy, 2004). Whereas direct interactions with brood parasites have been shown to increase host nest vigilance on the following day (Feeney & Langmore, 2015), it remains unknown whether referential alarm calls also convey the ongoing risk of parasitism, thereby modifying the future behaviours of hosts until or at the time of day when parasitism is most likely to occur. Yellow warblers (*Setophaga petechia*, hereafter 'warblers') express a suite of anti-parasitic behaviours, including referential signals (Gill & Sealy, 2004; Lawson et al. 2020a). These 'seet' calls refer specifically to brood parasitic brown-headed cowbirds (*Molothrus ater*, hereafter 'cowbirds'), whereas 'chip' calls generically alert mates to diverse predators and intruding conspecifics (Gill & Sealy, 2004; Lawson et al., 2021a). In response to experimental exposure to either parasitic stimuli (e.g. live or model female cowbirds, playbacks of cowbird calls) or referential seet calls, female warblers return to sit tightly on the nest (Figure 16; Gill & Sealy, 2004). That is, conspecific seet calls alone elicit the same immediate defensive response that live cowbirds do. To date, published experiments have been performed during daylight hours, when the actions of warblers may prevent cowbirds from examining nest contents and removing host eggs (reviewed in Lawson et al., 2021b). Yet, such a response does not prevent parasitism per se because cowbirds typically lay their eggs at dawn, just before sunrise Neudorf & Sealy, 1994). Could the detection of seet calls during the day also signal future parasitism risk, leading female warblers to engage in vigilance at dawn on the following day?

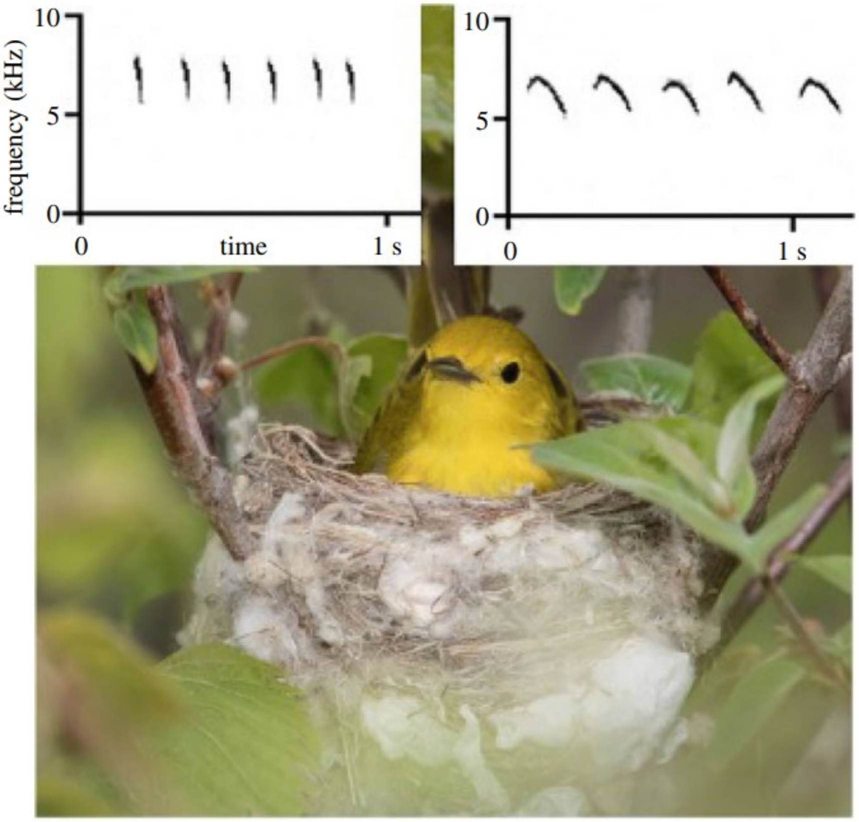

**Figure 16.** Female yellow warbler on the nest (photo credit with permission: M. Dunn of Roads End Naturalist); insets: spectrograms of chip (left) versus seet (right) calls with frequency (y-axis) and time (x-axis) indicated.

The ability to use personal experiences from the past to alter current or immediate future behaviour occurs across diverse animal lineages (e.g. Dere et al., 2018). In yellow warblers, differences in the meaning of referential seet calls versus generic anti-predator chip calls provide us with the opportunity to test longer term impacts of current communication signals in two divergent reproductive contexts: brood parasitism risk (seets) versus generic territorial and nest-predation threats (chips). We hypothesize that the perception of referential seet calls involves the mental representation of the parasitism-specific threat (Gill & Bierema, 2013) and here we test whether the detection of seet calls alters future vigilance of females specifically at the time when increased nest attendance would be most adaptive. By contrast, no specific pattern of altered future behaviour is expected in response to the generic chip alarm calls; as a general threat signal, chip calls should not predictably alter vigilance at a specific (future) time of day.

## 4.3 MATERIALS AND METHODS

During May and June in 2020 and 2021, we conducted sequential daily playback experiments and early morning observations at nests (N = 27) of unmarked yellow warbler pairs, located at three study sites (greater than or equal to 5 km apart) in east-central Illinois, USA. Here, cowbird parasitism rates on warbler nests vary between 30 and 40% (Lawson et al., 2021a). For three consecutive days during the first half of the incubation period, we exposed nesting pairs between 8.00 and 10.00 h to a single 10 min daily treatment. We collected partial data from three nests owing to depredation between visits. All but two nests received a silent control (no playback) on Day 1 (the other two received them on Day 3) and we then randomly assigned seet or chip call playbacks on Days 2 and 3. For call treatments, we broadcast a randomly selected exemplar at a sound level of approximately 70 dB (from 1 m) from a FOXPRO NX4 game caller (Lewistown, PA, USA), placed approximately 10 m from the nest on the ground. We used five unique, 10 min exemplar files of each call type, which were each composed of calls from 2 or 3 different individuals interspersed with 2–6 s of silence; all were sourced from our previous study (Lawson et al., 2020a) and filtered out below 0.5 kHz, which is much lower in range than either of our stimulus types (Figure 17).

The following morning (n = 74 nest-mornings total), we recorded female nest attendance data for 80 min (20 min before to 60 min after local sunrise). We note that we did not collect behavioural data for this study immediately following or during the course of the day of each playback. We quantified pauses in the female's incubation behaviour by tallying the number of times she left and by the total duration that she was off the nest per observation period. Observations were conducted directly by personnel situated at a distance of greater than or equal to 5 m (n = 51 nest-mornings) and/or by a set of Thermochron iButton (Whitewater, WI, USA) temperature loggers placed inside the nest cup (n = 46 nest-mornings) (following Ospina et al., 2018). Females were scored as off-nest when observed flying away or when iButton temperatures decreased by more than 1°C relative to the temperature fluctuations recorded by a second iButton positioned approximately 1 m below the nest. Females were scored as back on the nest when they were seen returning or iButton temperatures increased by more than 1°C. We based these iButton criteria on the positive relationship between our two metrics of nest attendance (times off the nest: Spearman's $\rho = 0.71$, $p = 0.0002$; duration off the nest: $\rho = 0.56$, $p = 0.007$) from mornings when both data collection methods were employed simultaneously (n = 23 nest-mornings at N = by more than 10 nests). When both collection methods were used at nests, we analysed data collected by the method employed during each of three mornings and the observation data when both methods were available for the full experimental extent. During visual observations, we also recorded whether males attended to females by visiting and, typically, feeding them on the nest.

We combined our two direct response metrics (i.e. the number of departures from and cumulative time off the nest) through a principal component analysis, extracting the first PC score (PC1: both eigenvectors = 0.71, eigenvalue = 1.2, variance explained = 61%) to represent the females' morning nest departure behavioural responses to playbacks. To assess the effect of treatment (silence, chip or seet) on nest attendance during the following morning, we used a general linear mixed model (GLMM) with playback type and experimental order as predictors, PC1 as the response variable and nest ID as a random effect. We assessed post hoc differences between playbacks using corrected Student's t-test p-values. Finally, we examined whether male nest visitation itself was related to female nest departure behaviours (PC1), playback treatment and the order of experimentation, with nest ID as a random effect. Parametric statistical tests were conducted in JMP 12.0 (SAS Inc., Cary, USA) while non-parametric tests were calculated using an online calculator (https://www.socscistatistics.com/tests/ spearman/). We set α = 0.05 for all analyses.

## 4.4 RESULTS

Female warblers’ nest departure behaviours (PC1) on the mornings following playbacks on the previous day varied by playback treatments (F2,46= 7.8, p = 0.0012) but not with experimental order (F1,46= 1.2, p = 0.28). Post hoc comparisons revealed fewer nest departures following seet calls (mean ± s.d.: −0.57 ± 0.75) versus both chip calls (0.27 ± 1.26; t46= −3.9, p = 0.0003) and silence (0.24 ± 1.06; t46= −2.1, p = 0.043), and no difference between chip calls and silence (t46= 0.87, p = 0.39) (Figure 17).

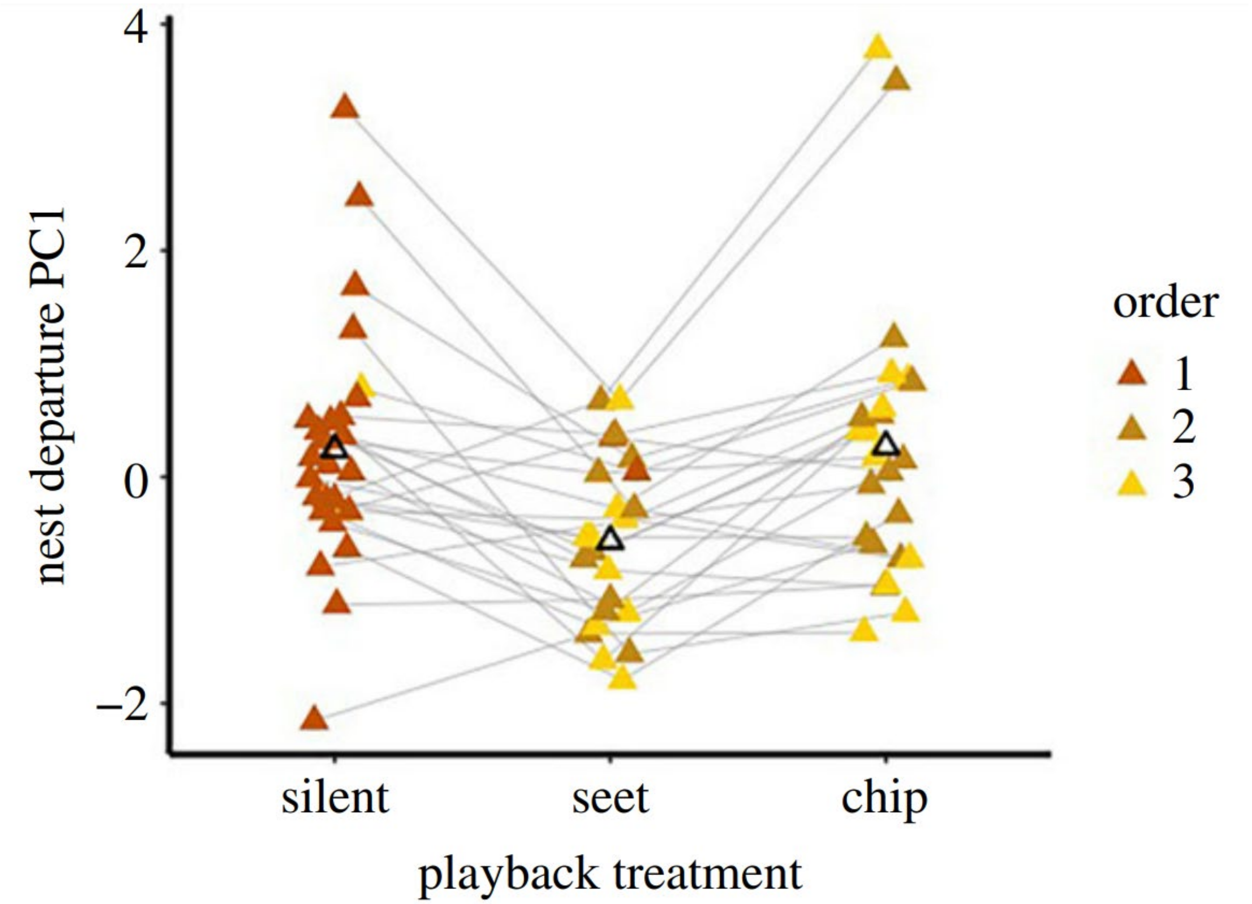

**Figure 17.** Female yellow warblers showed reduced nest departure behaviours (PC1) on the morning following playbacks of referential seet calls than following either chip calls or silent controls; lower values of PC1 reflect more time spent on and fewer departures from the nest. Each grey line connects data points sourced from the same nest; the playback treatment's order (order) of presentation is indicated by the different colours of the jittered data points. The empty black triangles indicate the mean for each treatment. Full and partial datasets per nest are all included in this figure.

For the subset of nest-mornings ($n = 50$) for which visual observations were made, male nest visitation was not statistically related to playback treatment ($F_{2,37} = 1.2$, $p = 0.32$) or experimental order ($F_{1,40} = 2.4$, $p = 0.13$), but was negatively related to female nest departures (with the regression equation as: $PC1 = 0.54 - 0.16x$ male nest visits; $F_{1,38} = 10.7$, $p = 0.0023$).

## 4.5 DISCUSSION

Yellow warblers are one of a handful of host species known to have referential calls denoting the presence of brood parasites around the nest (Gill & Sealy, 2004; Feeney & Langmore, 2015]. When female warblers detect seet calls, they respond immediately with cowbird-specific nest-protection behaviours (Gill & Sealy, 2004; Figure 1). Here, we discovered that females also perceived these calls to provide information about future risk at dawn when female cowbirds lay their eggs. We detected more intensive nest attendance (fewer and shorter nest departures; PC1) on the morning after playbacks of referential seet calls relative to generic chip calls or silent controls (Figure 2). Specifically, female warblers left less often and spent less total time off their nest following the previous day's experimental exposure to seet calls. Thus, these antiparasitic calls conveyed additional meaning about parasitism risk, causing warblers to be more vigilant at the nest during the following morning at the time when parasitism may occur. In turn, higher female nest attendance was statistically linked to more male at-nest (feeding) visits, but the males' behaviour was not caused by the experimental treatments. Finally, we did not assess in this study whether fewer nest departures would in fact effectively reduce parasitism by blocking female cowbirds' assessments of nest content (Strausberger, 1998) or their access to lay into the nest (Ellison & Sealy, 2007) in the early mornings.

We propose that female yellow warblers possess the capacity to respond to referential alarm calls adaptively by altering their behaviours to face future threats of brood parasitism. The time elapsed between our alarm call playbacks on one day and the response patterns detected on the next morning at the nest suggests that female warblers have either continued to engage in or specifically recalled events that occurred in the past (the day before) when making decisions to stay in or leave their nests the following dawn. These patterns of behaviour could result from lower level cognitive mechanisms (e.g. semantic memory, instinct) and ongoing (afternoon and overnight) heightened motivation to sit longer on the nest following seet call playbacks. Alternatively, yellow warblers may specifically engage in mental time travel, recalling the past event of detecting seet calls to influence nest vigilance on the next day (Neudorf & Sealy, 1994; Clayton & Dickinson, 1998). Mental time travel requires complex cognitive capabilities, including episodic-like memory as well as the ability to act outside of current motivational state (Clayton & Dickinson, 1998). Some argue (Baciadonna et al., 2020) against the latter ability in animals, suggesting that animals cannot anticipate or plan for future events. Although the present study does not address the specific cognitive mechanisms involved, our experimental design minimized possible differences in reproductive stage-relevant breeding motivation by testing warbler nests solely during early incubation, a time when female hosts are mostly on their nests to incubate their eggs. Additionally, we played the two types of alarm calls in random order to the same hosts on successive days to avoid treatment bias in daily motivational differences as incubation progressed. Nevertheless, further experimentation should specifically aim at understanding whether the decision to protect the nest at dawn in response to seet calls the day before involves the recollection of distant past events and the capacity to flexibly plan for the future. Specifically, do cues from parasitic cowbird calls on one day alter ongoing nest vigilance until the next morning or do they cause a specifically timed future vigilance by female warblers at the next dawn? Could responses to seet call or cowbird stimuli persist even further into the future? Is heightened nest attendance on the morning after a cowbird or seet call encounter adaptive to effectively preventing parasitism? Answers to these questions would help to establish the cognitive mechanisms and selective pressures underlying the decisions of female yellow warblers to engage in nest vigilance.

Studies of episodic-like memory of mental time travel have primarily focused on foraging contexts (e.g. Clayton & Dickinson, 1998), yet reproductive contexts also provide rich opportunities that explore these concepts. For example, captive brood parasitic brown-headed cowbirds engage in complex cognition consistent with mental time travel within the context of their own egg-laying decisions (White, 2020). We, therefore, propose both that our data on female yellow warbler responses to anti-parasitic seet calls be replicated and that the birds' responses be studied across multiple time points following playbacks to explore more fully the nature and timecourse of their referential alarm calls and anti-parasitic responses.

# CHAPTER 5: HETEROSPECIFIC EAVESDROPPING ON AN ANTI-PARASITIC REFERENTIAL ALARM CALL

## 5.1 ABSTRACT

Referential alarm calls occur across taxa to warn of specific predator types. However, referential calls may also denote other types of dangers. Yellow warblers (*Setophaga petechia*) produce “seet” calls specifically to warn conspecifics of obligate brood parasitic brown-headed cowbirds (*Molothrus ater*), which lay their eggs in the warblers’ and other species’ nests. Sympatric hosts of cowbirds that do not have referential alarm calls may eavesdrop on the yellow warbler’s seet call as a warning system for brood parasites. Using playback presentations, we found that red-winged blackbirds (*Agelaius phoeniceus*) eavesdrop on seet calls of yellow warblers, and respond as much to seet calls as to cowbird chatters and predator calls. Red-winged blackbirds appear to eavesdrop on seets as warning system to boost frontline defenses on their territories, although they do not seem to perceive the warblers’ seets as a cue for parasitism per se, but rather for general danger to the nest.

## 5.2 INTRODUCTION

Vocal communication involves information transfer through auditory cues from a sender to receiver (Bradbury & Vehrencamp, 2011). Acoustic and other non-private signals can be eavesdropped upon by unintended receivers, both conspecific or heterospecific (Wiley, 1984). Heterospecific eavesdropping on vocal signals is common across many species of birds and mammals (Magrath et al., 2015), and can provide benefits such as the earlier detection of predators, increased foraging opportunities, and better informed decision-making for habitat selection or predator avoidance (Magrath et al., 2015; Batcheller, 2017; Mönkkönen and Forsman, 2002). Eavesdropping on heterospecifics can provide more or different types of information than conspecific eavesdropping alone because interspecific differences in sensory abilities and in space use within habitats likely expand the sensory-space covered by a single species (Seppänen et al., 2007; Goodale et al., 2010).

Eavesdropping on heterospecific alarm calls, or vocalizations that alert others of nearby predation risk (Sherman, 1977), has been detected across diverse lineages of birds and mammals (Magrath et al., 2015), and provides eavesdroppers with general information about predatory threats. Referential alarm calls in particular can indicate which of a suite of threats is present, each requiring specific actions to evade (Evans et al., 1993; Oda & Masataka, 1996; Evans, 2004; Rainey et al., 2004a, b; Suzuki, 2012; Zuberbühler, 2009). Referential alarm calls are vocalizations that denote specific objects, and elicit particular behavioral responses from animals that hear these calls (Evans et al., 1993). For example, Verreaux's sifakas (*Propithecus verreauxi*) produce different referential alarm calls for aerial vs. terrestrial predators, and listeners respond with specific antipredatory responses depending on the predator type referenced (Fichtel & Kappeler, 2002), which likely increases survival and imparts fitness benefits (Gill & Bierema, 2013).

Con- and heterospecific eavesdropping on referential alarm calling is particularly widespread in songbirds, and is well-studied in the context of improving nest defense and minimizing nest detection (Gill & Sealy, 2003; Gill & Sealy, 2004; Platzen & Magrath, 2005; Davies et al., 2005; Haff & Magrath, 2012; Suzuki, 2015; Yu et al., 2017). In turn, threats to nests include both nest predators that depredate eggs and nestlings, and brood parasites that forgo nest building and instead lay their eggs in the nests of others, leaving the host adults to care for the foreign egg(s) and chick(s) (Davies, 2010). To protect the nest from these threats, many species act aggressively toward both predatory and parasitic intruders within their territories/near their nests (Welbergen & Davies, 2009; Kilner & Langmore, 2011; Feeney et al., 2012; Feeney & Langmore, 2012).

In the context of anti-parasitic nest defense, success hinges on early detection of brood parasites prior to their discovery of the host's nest (Sealy et al., 1998). Eavesdropping on alarm calls that signal brood parasites could have several possible benefits, including increased individual vigilance, social mobbing, and, eventually, decreased parasitism, especially for species that do not have a referential system of their own. Therefore, it is hypothesized that eavesdropping on heterospecific referential alarm calls that signal brood parasitic threats should evolve. Prior work has identified parasite-specific alarm calls in diverse host–parasite systems (Feeney & Langmore, 2015; Wheatcroft & Price, 2019), and as predicted, the first evidence for heterospecific recognition of these calls was recently described across two species of congeneric *Acrocephalus* reed warbler hosts of the common cuckoo (*Cuculus canorus*) that use acoustically similar calls to signal the parasite's presence (Yu et al., 2019). However, none of these calls fit the full definition of referential alarm calls, as their elicited responses include general recruitment and mobbing, but not specifically anti-parasitic defenses.

The yellow warbler (*Setophaga petechia*) is the only known species to use referential alarm calls to signal the presence of a brood parasite (Gill & Sealy, 2004). In response to obligate brood parasitic brown-headed cowbirds (*Molothrus ater*, hereafter "cowbirds"), yellow warblers of both sexes produce a "seet call" that alerts their pair-bonded mates of the brood parasitic threat (Gill et al., 2008), and uniquely, females do not recruit to mob the parasitic threat but instead return to and sit tightly on their nest, therefore physically reducing brood parasitism risk. Seet calls are only produced in response to cowbirds, and almost exclusively during laying and incubation when the nest is at the highest risk of parasitism (Gill & Sealy, 2004; Wheatcroft & Price, 2019; Gill et al., 2008). Yellow warblers thus present an exceptional system to study whether any other hosts of brood parasites eavesdrop on heterospecific referential alarm calls to boost their own nest defenses toward cowbirds.

Here we present new evidence that red-winged blackbirds (*Agelaius phoeniceus*, hereafter "redwings"), another common host of brown-headed cowbirds (Searcy & Yasukawa, 2014; Strausberger, 2001; Shaffer, 2003) which are phylogenetically distant and vocally distinct from yellow warblers, actively eavesdrop upon and respond to seet calls, and may thus potentially use neighboring yellow warbler calls as an early warning system against brood parasitism risk. Redwings often nest in loose aggregations with yellow warblers where closer proximity to redwing nests is correlated with a decrease in parasitism rates of the nearby warblers (Clark & Robertson, 1979). This may be because redwings frontload their nest defenses, meaning that they use both vocal and physical aggression toward cowbirds to prevent them from accessing and parasitizing the nest (Gill et al., 2008; Robertson & Norman, 1976, 1977; Ortega & Cruz, 1988; Neudorf & Sealy, 1992; Gill et al., 1997a; Strausberger & Horning, 1997; Cruz, 1999; Yasukawa et al., 2016; Louder et al., 2020). However, redwings do not have a known referential alarm call system of their own, and as such, redwings may eavesdrop on yellow warblers' "seet calls" to enhance their own nest defense against cowbirds.

To assess whether redwings use yellow warbler seet calls as a frontline defense against cowbird parasitism, we report on three analyses from two playback experiments. The first experiment was conducted on yellow warbler territories for a separate study, but that also comprised heterospecific (including redwing) data. The second experiment sought to directly test redwings' responses to the playback types. In each experiment, we broadcast seet calls, cowbird, and nest predator vocalizations, as well as procedural controls at focal individuals. For the first experiment, we broadcast playback treatments on yellow warbler territories and tested the hypothesis that redwings respond similarly to cowbird chatters and warbler seet calls, but not to the other warbler alarm call (the generic "chip") or other heterospecific vocalizations from neighboring territories. For the second study, we located active redwing nests to investigate the hypothesis that territorial redwings respond to seet calls to enhance their frontloaded nest defenses against cowbirds. We predicted again that redwings' responses to seet calls would be similar to cowbird chatters, but not to other heterospecific calls. For the final analyses, we were interested whether in the second experiment, the redwings' distance to the closest yellow warbler territory influenced redwing responses (specifically, calling rates) toward playbacks of cowbird chatters and seet calls. If redwings actively eavesdrop on seet calls as a warning signal for brood parasitism, and seet calls are perceptually equivalent to chatters, then redwings nesting closer to yellow warbler territories are predicted to mount stronger responses (more calls) to playbacks of cowbird chatters and seet calls than redwings that nest farther away from yellow warblers and thus do not have access to the heterospecific hosts' signal.

## 5.3 METHODS

### 5.3.1 Sites and study species

Both playback experiments (described below) took place in multiple wetlands in Champaign (n = 3), Iroquois (n = 1), and Vermilion counties (n = 3) in central Illinois, USA. Sites were comprised of mesic shrubland habitat, with dominant shrubs including willow (Salix spp.), dogwood (Cornus spp.), and Autumn Olive (Elaeagnus umbellate), with mesic grasses abundant among shrubs. Patches of cattails (Typha spp.) and reed (Phragmites spp.) were prevalent along bodies of water at most sites (Kelly, 2017; Kelly et al., 2018).

Redwings are sympatric with yellow warblers in Illinois, and both are parasitised by cowbirds (Lowther et al., 1999; Yasukawa & Searcy, 2019; Merrill et al., 2017, pers. obs.). At our sites, redwings arrive as early as February and nest from late-April through late-July, with peak breeding in late-May (Kelly, 2017; Kelly et al., 2018; pers. obs). Redwings are polygynous, and males may have several nests from different females on their territory[34]. Yellow warblers arrive at our sites late-April and breed from early-May through late June, with peak breeding mid-to-late-May. At these sites the interspecific overlap of territories was common between redwings and yellow warblers (pers. obs.).

### 5.3.2 Playback stimuli construction

For our experiments, we constructed playlists for six different playback treatments: (1) female cowbird chatters (brood parasite), (2) yellow warbler seet calls (cowbird-specific anti-parasitic alarm call (Gill & Bierema, 2013; Gill & Sealy, 2003; Gill & Sealy, 2004; Sealy et al., 1998; Gill et al., 1997a), (3) yellow warbler chip calls (general antipredatory alarm call, Gill et al., 1996, Hobson et al., 1988), (4) redwing chatters (general conspecific vocalization, Knight & Temple, 1988), (5) blue jay calls (a warbler and redwing nest predator, Smith et al., 2013), and (6) wood thrush songs (an innocuous heterospecific control that is sympatric with redwings but do not prey, parasitize, or compete with them, Kelly et al., 2019). We included blue jay calls to tease apart if redwings responded to seet calls as a general alarm call, or a referential alarm call specifically informing brood parasitism risk. Using both a brood parasite and predator call presentation is necessary to fully understand whether the host's aggressive responses are specifically anti-parasitic or general (Rothstein & Robinson, 1998). The chip was chosen as a relevant general alarm stimulus for playbacks on yellow warbler territories, and redwing chatters were used as a relevant territory intrusion stimulus on redwing territories. Note that experiments 1 and 2 differed in which treatments were used (described below).

We used audio files from Xeno Canto, all sourced from the Midwestern and Southwestern United States (Colorado, Illinois, Michigan, Minnesota, and Ohio), except for seet calls, which were sourced directly from Gill and Sealy (2003), and redwing chatters, which were sourced from Lynch et al. (2018). Playlists were created using Adobe Audition CC 2019. To avoid pseudoreplication (Kroodsma et al., 2001), we constructed five different playlist files for each treatment, and chose one exemplar file randomly for each playback trial (described below). Each playlist was comprised of vocalizations from at least three different individuals. Vocalizations from individuals were placed in a random order, and then repeated to obtain the 10-min playlist. Intervals of silence were placed into between vocalizations, with intervals ranging from 2 to 6 s based on rates found in natural recordings on Xeno Canto. Amplitude was adjusted such that sounds played from our speaker at full volume were ~90 dB (measured 0.5 m from speaker). To minimize signal-to-noise ratio in playback files, frequencies below 500 Hz, which are well below the range of any of our stimuli, were filtered out.

For both experiments, playbacks were conducted with an AYL-SoundFit speaker connected to a Samsung Galaxy 8 cellular phone with the audio files. We placed equipment ~1 m high in vegetation and recorded data from > 10 m away. Playback trials occurred for 10 min. For both experiments, we retested each territory 24–72 h later (mean = 41) to avoid habituation, with a different, randomly assigned treatment to prevent order effects. All statistical tests were conducted in the statistical program R 3.6.1 (packages lme4, nlme, multcomp, emmeans and car; see "Statistical analyses" section), with $\alpha = 0.05$.

These studies were approved by the animal ethics committee (IACUC) of the University of Illinois (#17259), and by USA federal (MB08861A-3) and Illinois state agencies (NH19.6279).

### 5.3.3 Experiment 1: playback on yellow warbler territories

Playback experiment: To assess if redwings respond similarly to cowbird chatters and seet calls, but not to other yellow warbler or heterospecific calls, we first used data collected during playback trials at active yellow warbler territories. Warbler territories were determined to be active in two ways: (1) if a nest with eggs was found on the territory, (2) if a nest could not be found but both a male and female were present on the territory on checks across multiple days and the pair produced alarm calls at the experimenter, which is indicative of nesting investment on the territory (Knight & Temple, 1988, pers. obs.). We also excluded any yellow warbler pairs seen carrying nesting material or insects, which signify building and nestling stage, respectively. Seet calls are almost exclusively produced during laying and incubation stage (Gill et al., 2008; Gill & Sealy, 1996), thus we only presented playbacks on territories presumed to be in those stages. Yellow warbler playbacks trials occurred from mid-May to late June in 2018 and 2019, and between 0500 and 1200 h local time. We did not systematically band territorial birds at our sites for individual identification prior to experimentation. Therefore, all nests tested were ≥ 30 m apart to maintain independence, as nests at this distance likely belong to different breeding units based on average territory size (Kelly et al., 2017; Kendeigh, 1941, DellaSala, 1986). In addition, we waited 30–60 min between playbacks at neighboring sites to avoid any carryover effects on neighbors.

Yellow warbler territories received one of five different playback treatments on two separate days, such that each territory was tested with two of the five playback types. Playbacks included cowbird chatters (n = 37), yellow warbler seets (n = 34), yellow warbler chips (n = 31), blue jay calls (n = 34), and wood thrush songs as an innocuous heterospecific control (n = 35), for a total of 171 playbacks. The playback speaker was placed 5–6 m from the yellow warbler male’s commonly used song post. During this set of initial playback trials, we specifically recorded a single binary response variable of whether any redwings responded to the playback or not. A response was only marked if one or more male redwings were present and alarm calling within 30 m (radial average distance of territory size, Kelly et al., 2019) of the speaker any time during the 10-min playback. Redwing alarm calls are described in Knight and Temple (Knight & Temple, 1988). We also report on which other species, apart from yellow warblers, responded to these same playback types during this experiment (see Supplementary Table 6).

### 5.3.4 Experiment 2: playback on red-winged blackbird territories and at nests

Playback experiment: To investigate if territorial redwings respond to seet calls to enhance their frontloaded nest defenses against cowbirds, we conducted playback trials at active redwing nests in 2018 and 2019. Playbacks were conducted using the same methodology and site locations in Champaign and Vermillion counties as Experiment 1. In Experiment 2, we increased the distance at which nests/song posts were tested, to maintain independence, to ≥50 m apart, as average territory size is larger for redwings than yellow warblers (Searcy & Yasukawa, 2014). This distance also prevented us from inadvertently testing the same parents twice at different nests, as redwings are polygynous harem breeders within their territories (Searcy & Yasukawa, 2014). Playbacks in 2018 were conducted 5 m from the male's focal song post. We were unable to search for nests in 2018, but used behavioral observations to select pairs that likely had an active nest (e.g., alarm calling, no nest material carried or fledglings present). In 2019, we instead placed speakers 5 m from known, located active nests and conducted playbacks during the incubation stage, as this is when cowbirds pose the gravest threat (Hobson & Sealy, 1990). In 2019, we conducted playbacks with speakers 5 m from focal nests, instead of song posts to reliably simulate the threats to the nest. We thoroughly searched sites 1–2 times weekly for active nests. Nest contents were checked every 3 days to ensure playback trials occurred during incubation.

Similar to experiment 1, redwings received one of five different playback treatments on 2 separate days, such that each pair was tested with two of the five playbacks: cowbird chatters (n = 5 in 2018; n = 18 in 2019), yellow warbler seets (n = 5 in 2018; n = 13 in 2019), redwing chatters (n = 9 in 2018, n = 16 in 2019), blue jay calls (n = 0 in 2018; n = 16 in 2019), and wood thrush songs (n = 6 in 2018; n = 17 in 2019), for a total of 105 playbacks across 2 years. In 2019, two nests were not retested as they were depredated between trials. For logistical reasons, we did not include blue jay calls in 2018. Only male responses were recorded in 2018 because only males responded to the playbacks on yellow warbler territories. After noting that females responded as well near known active nests, we also recorded responses for both sexes in 2019 (see "Statistics and reproducibility" below).

During the playback trial, we recorded the following behavioral responses from the target individual within 30 m of the speaker: (1) response latency (sec after the start of trial when a switch to aggressive behaviors occurred: posturing, hopping, alarm calling, or attacking the speaker); (2) closest approach to the speaker (m); and (3) call rate (total calls/10 min). In 2018, we only counted "checks" and "cheers" as these are general alarm calls used by redwings in many contexts (Beletsky et al., 1986). In 2019, we expanded to counting "checks", "chits", "chonks", and "cheers" as they are all used interchangeably as nest defense alarm calls by both sexes, although only males produce cheer calls (Knight & Temple, 1988; Beletsky et al., 1986; Yasukawa et al., 1986). Therefore, we analyzed call rate for 2018 and 2019 separately.

### 5.3.5 Calling rate analysis across distances between heterospecific territories

We used spatial data to evaluate if redwings nesting closer to yellow warbler territories mount stronger responses (calls) to playbacks of cowbird chatters and seet calls than redwings nesting farther away that do not access to the heterospecific hosts' signal. In 2019, we recorded locations of playbacks conducted at known, active redwing nests and yellow warbler territories using GPS units with 3 m accuracy (Garmin eTrex 10). Using software in ArcGIS (ver 10.4 ESRI), we measured the distance (m) of each redwing nest to the nearest playback conducted on a yellow warbler territory. We assumed that we found all active redwing nests and warbler territories within these study subsites. Prior work (Clark & Robertson, 1979) found that redwings would respond to playbacks up to 60 m from the nest, which coincided with the redwings' territory boundaries. Many of our territories in marginal, upland habitats were larger than the reported average, so we extended our cutoff range to count redwing nests that were up to 75 m away from a yellow warbler territory. If redwings were deriving any benefit from eavesdropping on cowbird danger as predicted by chatters and seets, then they would need to nest within this range from a yellow warbler nest to be able to eavesdrop on and respond to their neighbor's seets.

### 5.3.6 Statistics and reproducibility

Experiment 1: playback on yellow warbler territories: We ran a nominal logistic regression that analyzed the effect of playback treatment on whether redwings were absent or present during trials. We then ran post hoc Tukey pairwise comparisons between cowbird vs seet treatments, seet vs wood thrush, and seet vs chip treatments to account for multiple comparisons between playback type pairs.

Experiment 2: playback on red-winged blackbird territories and at nests: We evaluated whether playback treatment affected the three response variables of interest (latency, total alarm calls, and closest approach) using a separate model for each. For latency and approach, we combined the data from 2018 and 2019, but for call rates, we analyzed data separately for the two years because some call types were not counted in 2018 and only males were recorded that year (see above). We determined if redwing (males and females separately) responded immediately (latency of <1 s) or with some latency (≥1 s) and conducted a $\chi 2$ test on the ratios by treatment to determine if birds were more likely to respond immediately during particular playback treatments. We also ran a negative binomial generalized mixed model on the nonzero response latencies (# of seconds to respond) with playback treatment and date as fixed effects, and redwing nest site ID as a random effect. For alarm call and closest approach variables, we log transformed the data after adding a small constant and ran general linear mixed models. In each model, we included playback treatment and date as fixed effects, and redwing nest site ID as a random effect. For all three models, we ran post hoc Tukey tests to multiple compare treatment pairs of least-square means.

### Calling rate analysis across distances between heterospecific territories

We ran separate analyses of variance for males and females, which analyzed calling rate (calls per min) during seet and cowbird playbacks with distance from the focal redwing's nest to the nearest yellow warbler territory and the playback call treatment (seet vs. chatter) as fixed effects.

## 5.4 RESULTS

### 5.4.1 Experiment 1: playbacks on yellow warbler territories

Male redwing presence on yellow warbler territories differed significantly by playback treatment ($\chi 2 = 17.08$, $p < 0.001$; Figure 18). Based on post hoc analyses, we found that redwings were present on yellow warbler territories more often during seet calls trials (41%) compared with both chip calls (19%, $p = 0.037$) and wood thrush song (*Hylocichla mustelina*) controls (3%, $p < 0.001$). In contrast, redwing presence on yellow warbler territories did not differ between seet calls and cowbird chatters (46%, $p = 0.189$).

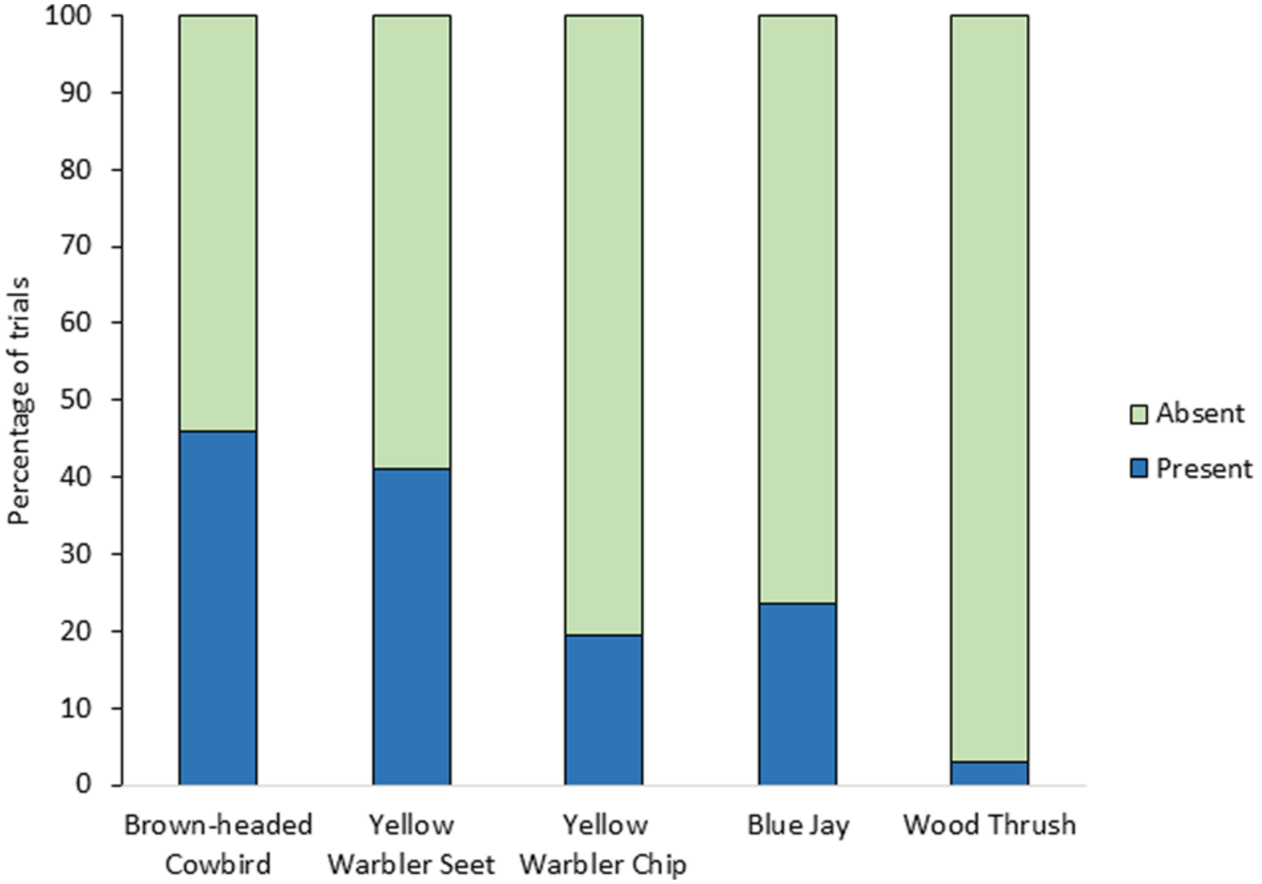

**Figure 18.** Percentage of trials for different playback treatments on yellow warbler territories in which at least one male redwing was present and responsive to the playback (dark blue) or absent (light green). Data were analyzed using a nominal logistic regression. Playbacks include cowbird chatters (n = 37), warbler seets (n = 34), warbler chips (n = 31), blue jay calls (n = 34), and wood thrush songs (n = 35).

### 5.4.2 Experiment 2: playback at red-winged blackbird nests

#### 5.4.2.1 Latency

Average latencies (including zeros) to respond to the treatments were highly variable for both redwing males (Figure 19) and females (Figure 20). The ratio of trials where male redwings responded immediately (latency of zero) to the playback differed significantly between treatments ($N = 104$, $\chi2 = 26.06$, $p < 0.001$). Based on post hoc pairwise comparisons of least-squares means, males were more likely to respond immediately to playbacks of cowbird chatters ($p < 0.001$), seet calls ($p < 0.001$), blue jay calls (*Cyanocitta cristata*, $p < 0.001$), and redwing chatters ($p < 0.001$), compared with wood thrush (control) songs. The ratio of trials where redwing females responded immediately (latency of zero) to the playback did not differ significantly between treatments ($N = 77$, $\chi2 = 7.23$, $p = 0.123$). Nonzero latencies also differed significantly between treatments for males ($F4,56 = 16.37$, $p < 0.001$) and females ($F4,59 = 16.13$, $p < 0.0001$). Based on post hoc pairwise comparisons of least-squares means, male redwings responded more quickly to cowbird chatters ($z = 5.12$, $p < 0.001$), seet calls ($z = 5.59$, $p < 0.001$), blue jay calls ($z = 5.18$, $p < 0.001$), and redwing chatters ($z = 6.25$, $p < 0.001$) compared with wood thrush songs. Female latencies showed the same pattern, where females responded more quickly to playbacks of cowbird chatters ($z = 5.72$, $p < 0.001$), seet calls ($z = 5.32$, $p < 0.001$), blue jay calls ($z = 4.54$, $p < 0.001$), and redwing chatters ($z = 7.45$, $p < 0.001$) compared with wood thrush songs. Latency to respond did not differ between any of the non-control playbacks for either sex (see Supplementary Table 7 for nonsignificant comparisons). Date of playback did not have a significant effect on nonzero latencies for males ($F1,103 = 0.372$, $p = 0.54$) or females ($F1,77 = 2.71$, $p = 0.108$) nor did year (males only) ($F1,56 = 0.36$, $p = 0.54$).

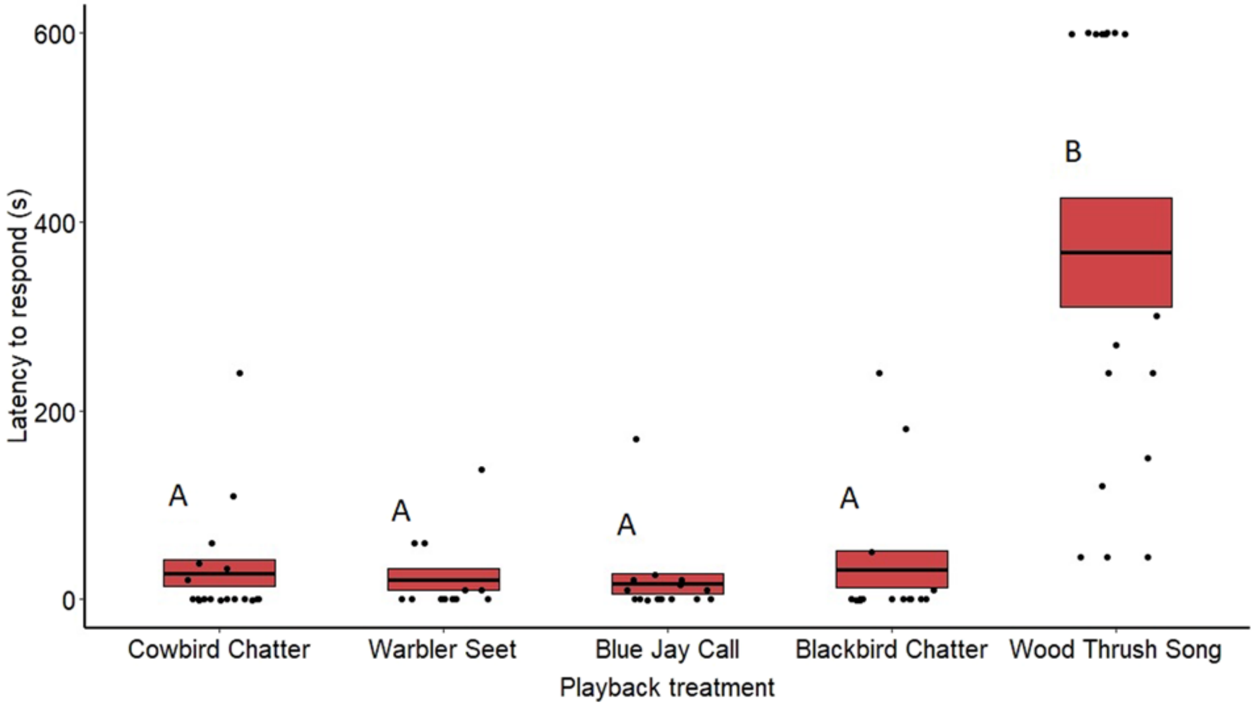

**Figure 19.** Latency for redwing males to respond to the playback treatments on redwing territories and at nests. Means are shown with the bold line, and shaded boxes represent standard errors. Data were analyzed using a zero-inflated negative binomial model. Boxes with different letters denote post hoc statistical differences between treatments (cowbird chatters n = 23, warbler seets n = 18, redwing chatters n = 25, blue jay calls n = 16, wood thrush n = 23). For p-values of post hoc comparisons, please refer to Supplementary Table 7.

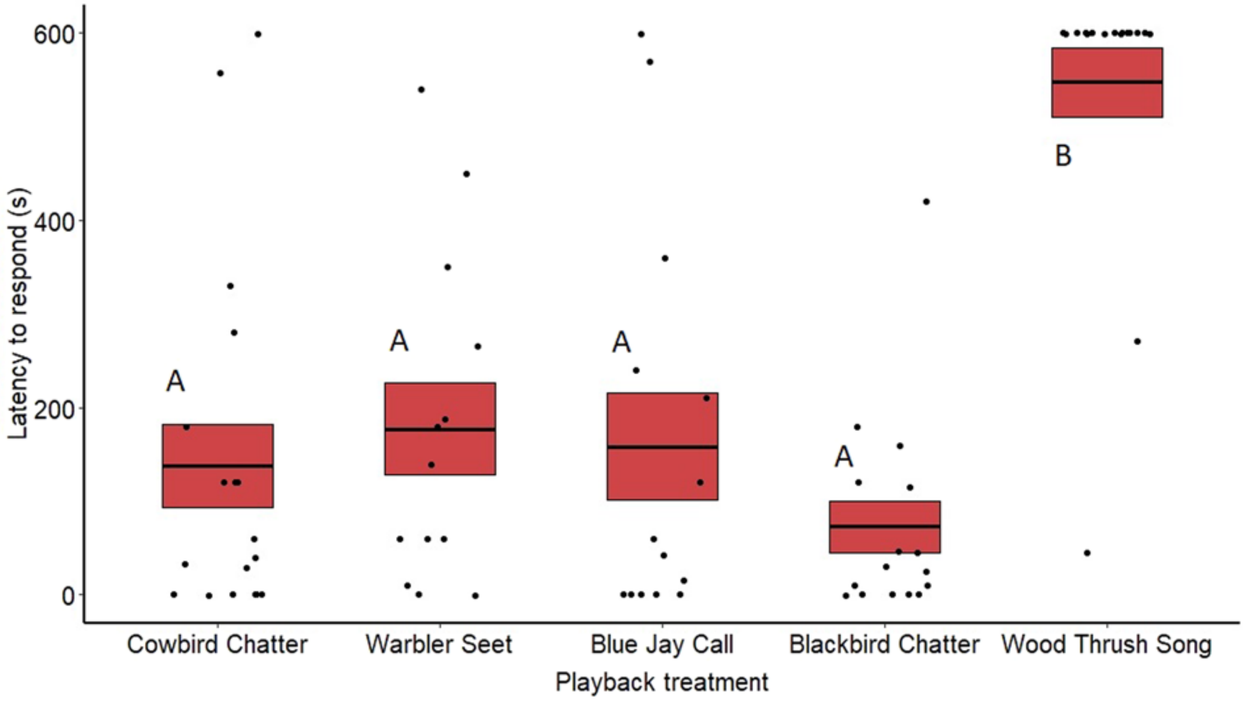

**Figure 20.** Latency for redwing females to respond to the playback treatments at redwing nests. Means are shown with the bold line, and shaded boxes represent standard errors. Data were analyzed using a zero-inflated negative binomial model. Boxes with different letters denote post hoc statistical differences between treatments (cowbird chatters n = 18, warbler seets n = 13, redwing chatters n = 16, blue jay calls n = 16, wood thrush songs n = 17). For p-values of post hoc comparisons, please refer to Supplementary Table 7.

#### 5.4.2.2 Closest approach

Closest approach also varied significantly between the treatments for both males (F4,104 = 10.25, $p < 0.001$) (Figure 21) and females (F4,77 = 3.68, $p < 0.01$) (Figure 22). Redwing males moved significantly closer to the speaker during redwing chatter playbacks compared with seets ($z = 4.18$, $p < 0.001$), blue jay calls ($z = -2.84$, $p = 0.03$) and wood thrush songs ($z = 5.81$, $p < 0.001$). Redwing males also approached the speaker more closely during cowbird playbacks than seets ($z = 2.90$, $p = 0.02$) and wood thrush songs ($z = 4.43$, $p < 0.001$). Females only approached more closely to playbacks of redwing chatters compared with the control wood thrush songs ($z = 3.57$, $p < 0.01$). All other pairwise comparisons were not significantly different (see Supplementary Table 8). Date of playback did not have an effect on male (F1,104 = 0.015, p = 0.90) or female approach (F1,77 = 0.65, p = 0.42), and neither did year (males only) (F1,104 = 0.911, p = 0.34).

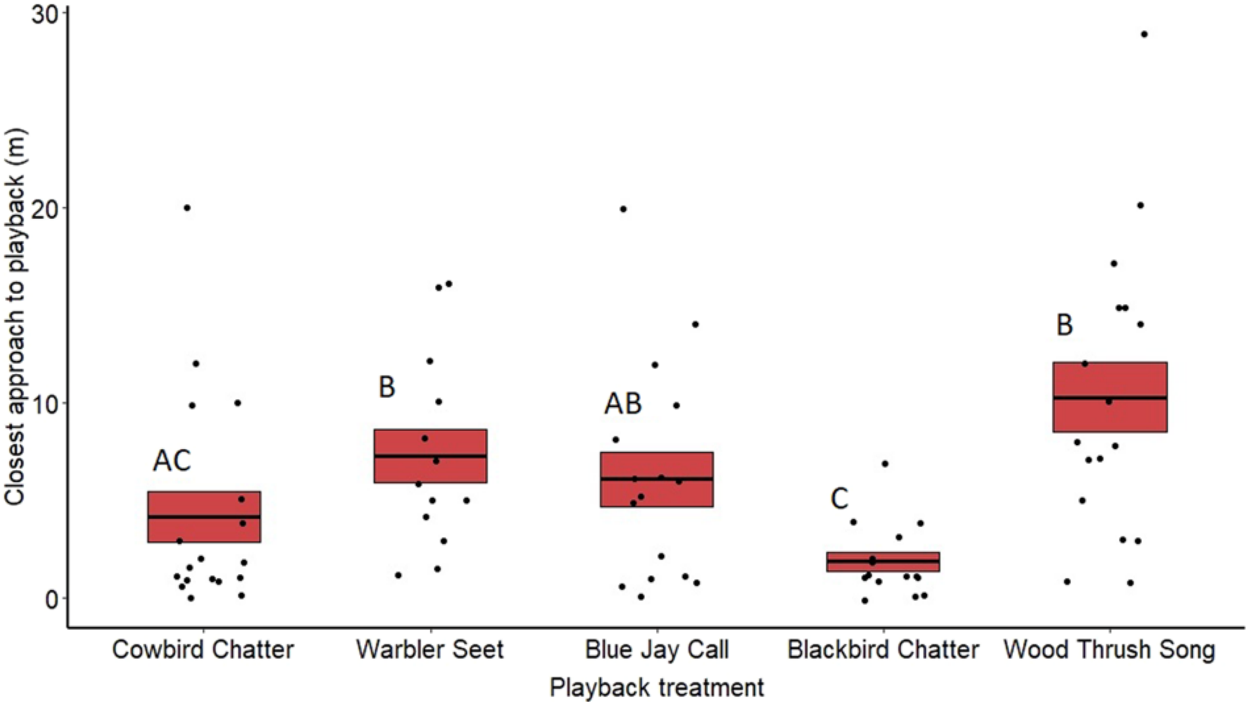

**Figure 21.** Closest approach to the playback speaker (in meters) by redwing males for the different treatments on redwing territories and at nests. Means are shown with the bold line, and shaded boxes represent standard errors. Data were analyzed using a general linear mixed model. Boxes with different letters denote post hoc statistical differences between treatments (cowbird chatters n = 23, warbler seets n = 18, redwing chatters n = 25, blue jay calls n = 16, wood thrush songs n = 23). For p-values of post hoc comparisons, please refer to Supplementary Table 8.

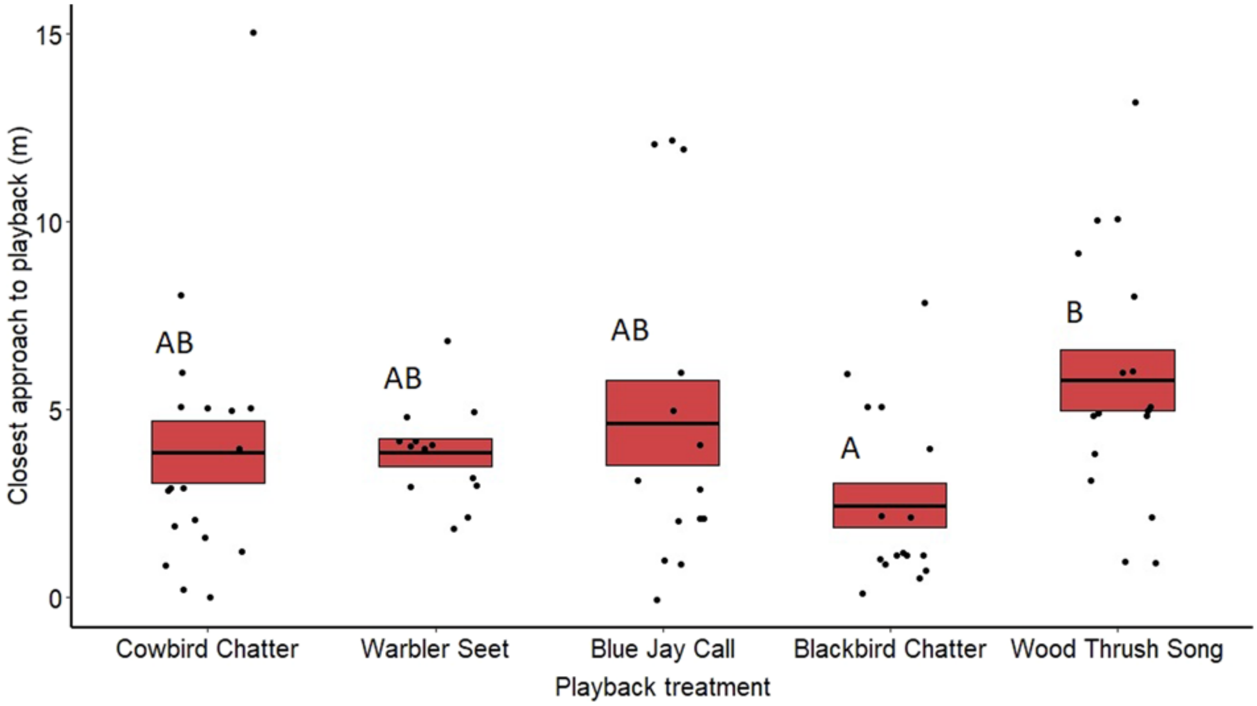

**Figure 22.** Closest approach to playback speaker by redwing females to respond to the playback treatments at redwing nests. Means are shown with the bold line, and shaded boxes represent standard errors. Data were analyzed using a general linear mixed model. Boxes with different letters denote post hoc statistical differences between treatments (cowbird chatters n = 18, warbler seets n = 13, redwing chatters n = 16, blue jay calls n = 16, wood thrush songs n = 17). For p-values of post hoc comparisons, please refer to Supplementary Table 8.

### 5.4.2.3 Calling rate

Rate of calling by redwing males differed significantly between treatments both in 2018 ($F_{3,24} = 16.53$, $p < 0.0001$) (Figure 23) and 2019 ($F_{4,78} = 25.81$, $p < 0.0001$) (Figure 24). Based on post hoc pairwise comparisons of least-squares means, in both 2018 and 2019 redwing males called more toward playbacks of cowbird chatters (2018: $z = -3.04$, $p = 0.01$; 2019: $z = -7.63$, $p < 0.001$), seet calls (2018: $z = -2.66$, $p = 0.03$; 2019: $z = -6.29$, $p < 0.001$), and redwing chatters (2018: $z = -8.16$, $p < 0.001$; 2019: $z = -9.76$, $p < 0.001$) compared with wood thrush songs. Redwings also called more toward playbacks of redwing chatter compared with cowbird chatter (2018: $z = 4.37$, $p < 0.001$; 2019: $z = 2.73$, $p = 0.04$), seet calls (2018: $z = -4.45$, $p < 0.001$; 2019: $z = -2.98$, $p = 0.02$), and in blue jay calls (2019: $z = 4.35$, $p < 0.001$). Calling rate did not differ significantly between cowbird and seet treatments in either 2018 (z = −0.27, p = 0.99) or 2019 (z = −0.44, p = 0.99) (see Supplementary Table 9). Date of playback had a significant effect on male calling rate in 2018 (F1,24 = 9.17, p = 0.01), where calling rate increased later in the season, however, this was not seen in 2019 (F1,78 = 1.78, p = 0.18).

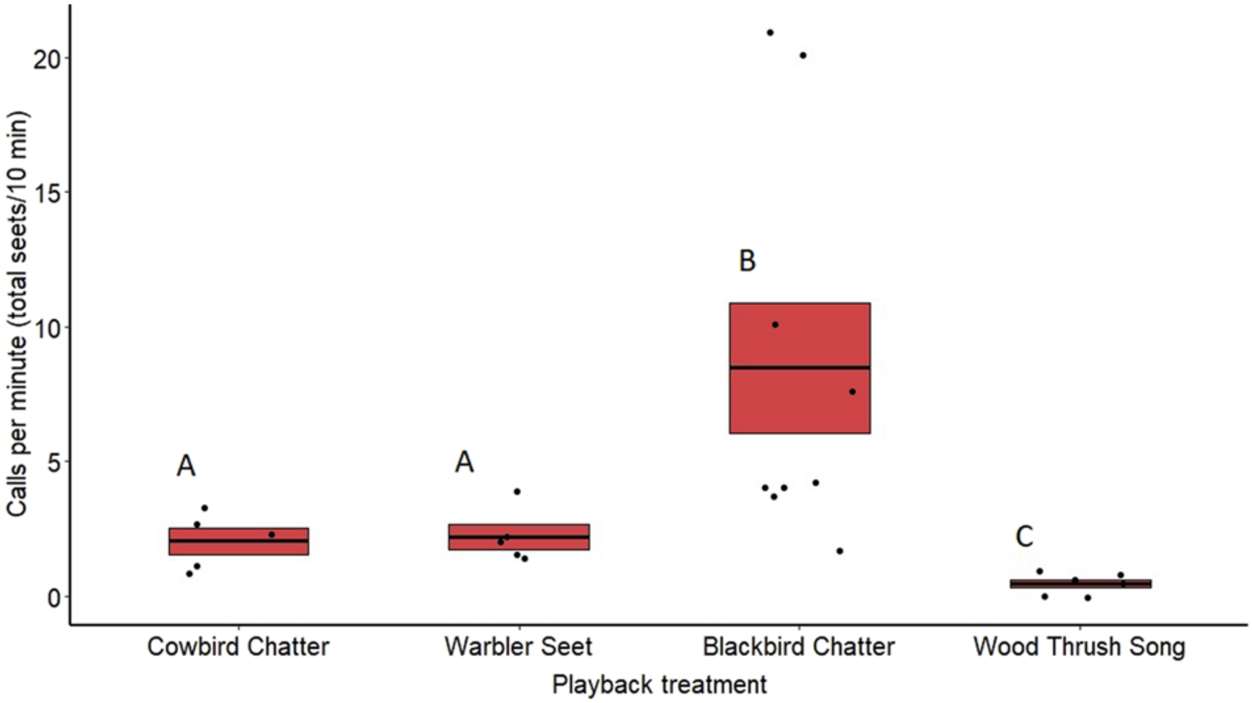

**Figure 23.** Average call rate for redwing males for treatments in 2018 on redwing territories. Means are shown with the bold line, and shaded boxes represent standard errors. Boxes with different letters denote post hoc statistical differences. Data were analyzed using a general linear mixed model. Boxes with different letters denote post hoc statistical differences between treatments (cowbird chatters n = 5, warbler seets n = 5, redwing chatters n = 9, wood thrush songs n = 6). For p-values of post hoc comparisons, please refer to Supplementary Table 9.

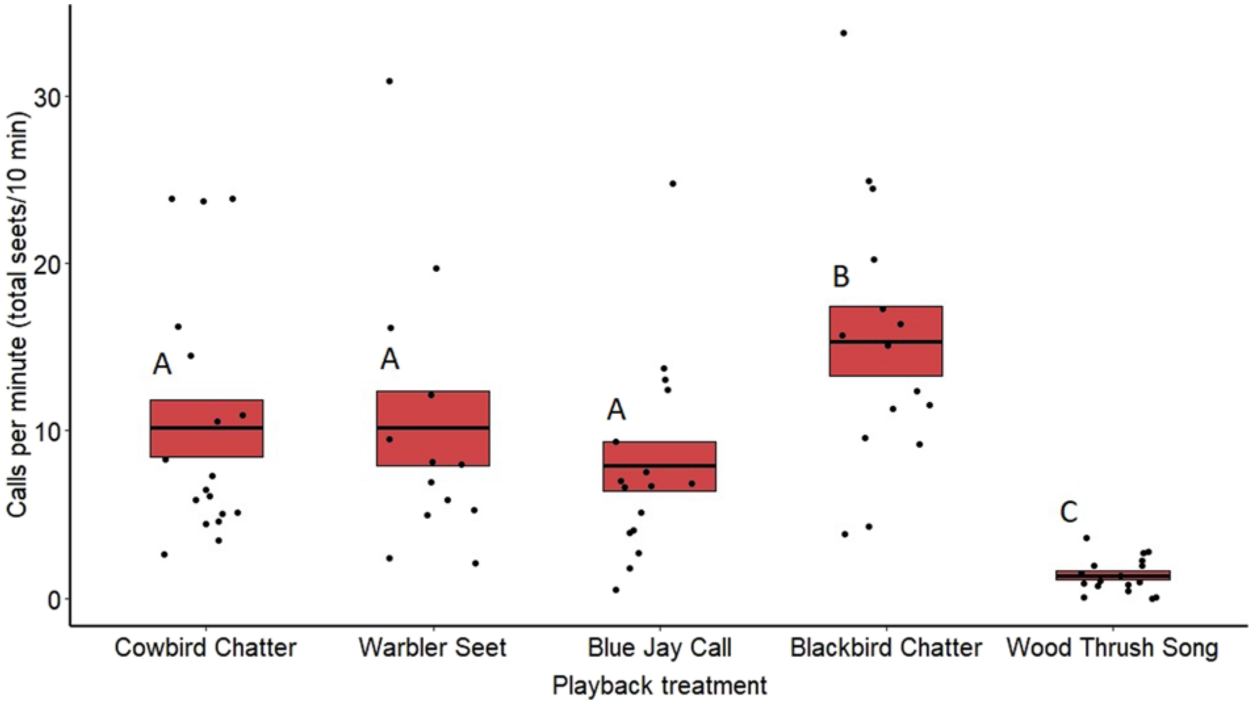

**Figure 24.** Call rate for redwing males in each treatment in 2019 at redwing nests. Means are shown with the bold line, and shaded boxes represent standard errors. Data were analyzed using a general linear mixed model. Boxes with different letters denote post hoc statistical differences between treatments (cowbird chatters n = 18, warbler seets n = 13, redwing chatters n = 16, blue jay calls n = 16, wood thrush songs n = 17). For p-values of post hoc comparisons, please refer to Supplementary Table 9.

Female calling rate also differed significantly between treatments ($F_{4,77} = 13.73$, $p < 0.0001$) (Figure 25). Similar to males, females called more toward playbacks of cowbird chatters ($z = -2.81$, $p = 0.039$), seet calls ($z = -4.42$, $p < 0.001$), blue jay calls ($z = -4.41$, $p < 0.001$), and redwing chatters ($z = -7.28$, $p < 0.001$) compared with wood thrush songs. Females also called more toward playbacks of redwing chatters compared with blue jay calls ($z = 2.76$, $p = 0.044$). Unlike males, calling rate for females was similar between redwing chatters and seet playbacks ($z = -2.45$, $p = 0.10$), and females called more during redwing chatter playbacks than cowbird chatters ($z = 4.69$, $p < 0.001$). Notably, females also did not differ in calling rate between the seet and cowbird treatments ($z = 1.99$, $p = 0.26$) (see Supplementary Table 9). Date of playback ($F_{1,77} = 0.30$, $p = 0.58$) did not have a significant effect on female calling rate.

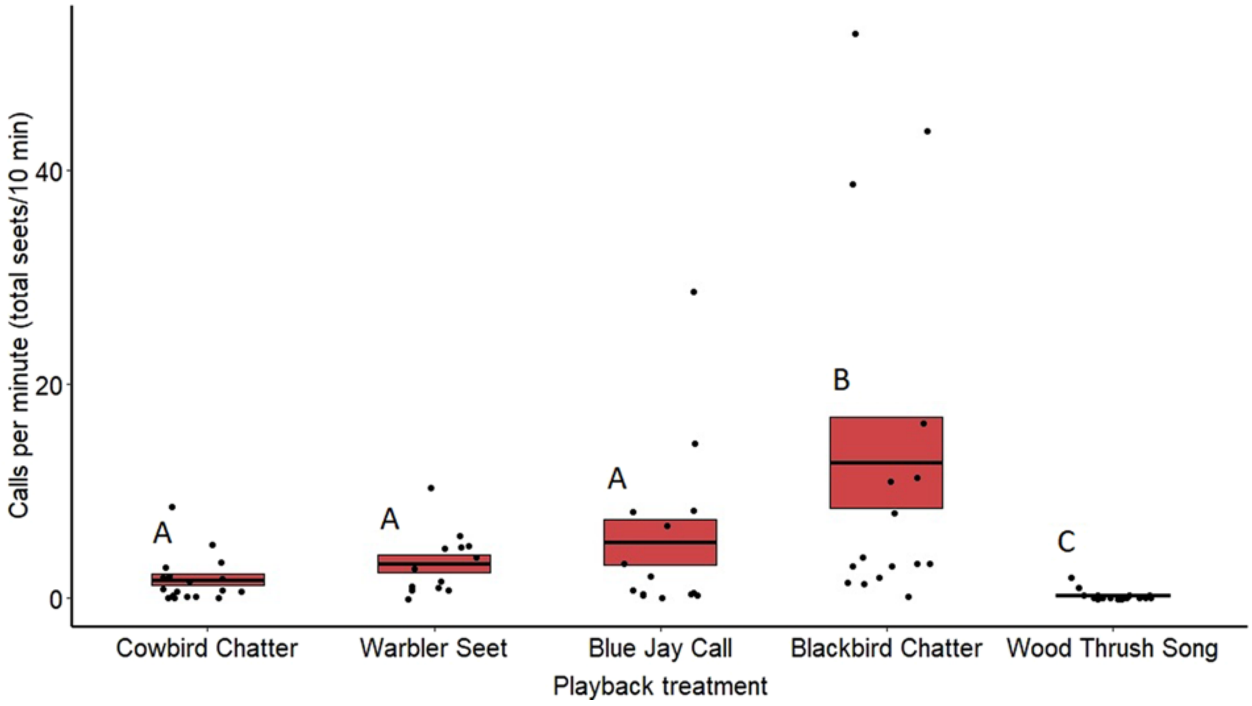

**Figure 25.** Call rate for redwing females for playback treatments at redwing nests. Means are shown with the bold line, and shaded boxes represent standard errors. Data were analyzed using a general linear mixed model. Boxes with different letters denote post hoc statistical differences between treatments (cowbird chatters n = 18, warbler seets n = 13, redwing chatters n = 16, blue jay calls n = 16, wood thrush n = 17). For p-values of post hoc comparisons, please refer to Supplementary Table 9.

### 5.4.2.4 Calling rate across distances between heterospecific territories

For male redwings, distance to nearest yellow warbler territory had a significantly negative effect on alarm-calling rate during cowbird and seet playbacks ($R2 = 0.24$, $F1,14 = 5.32$, $p = 0.03$; Figure 26): male redwings with nests further away from the nearest yellow warbler territory called less toward playbacks of seets and cowbird chatters. Playback treatment itself was not a significant predictor, in that the focal redwings' alarm responses to both cowbird chatters and seets showed a similarly negative pattern of nest/territory-distance dependence ($F1,14 = 0.51$, $p = 0.48$). We did not see this pattern for female response rates based on distance ($F1,13 < 0.001$, $p = 0.98$; Figure 27) or treatment ($F1,13 = 0.37$, $p = 0.55$).

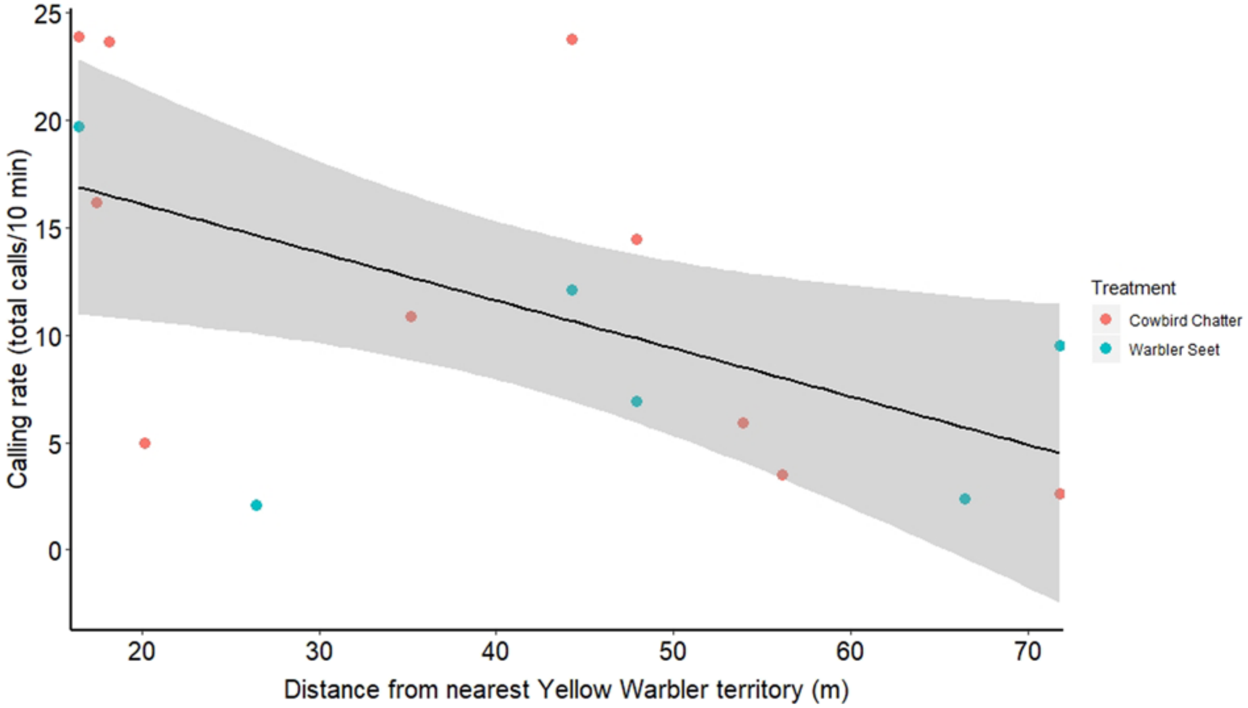

**Figure 26.** Calling rate of male redwings during seet and cowbird playbacks at redwing nests (2019) plotted along distance from the focal pair's nest to nearest yellow warbler territory (R2 = 0.26, F1,14 = 6.38, p = 0.02). Gray areas represent the 95% confidence interval of the slope. Treatments are marked with orange (cowbird) or blue (seet) coloring.

**Figure 27.** Calling rate of female redwings during seet and cowbird playbacks at redwing nests (2019) plotted along distance from the focal pair's nest to nearest yellow warbler territory (F1,13 = 0.25, p = 0.62). Treatments are marked with orange (cowbird) or blue (seet) coloring.

## 5.5 DISCUSSION

Our results demonstrate a series of patterns in which (1) both male and female redwings respond to the seet call as to other signals for danger to their nests, (2) both sexes of redwings show response equivalency between cowbird chatters and seet calls, (3) male redwings respond to anti-parasitic yellow warbler calls similarly to cowbird chatters, but not to yellow warblers chip calls, and (4) male redwings respond more to cowbird chatters and seet calls coming from closer yellow warbler neighbors. Taken together, these lines of evidence support that redwings eavesdrop on heterospecific referential alarm calls to enhance frontline defenses against parasitic cowbirds and other threats to their own nest.

In our first experiment, we found that nearly half of all cowbird chatters and seet call playbacks on yellow warbler territories drew in neighboring redwings. Redwings were as likely to respond to seet playbacks as they were to cowbird playbacks but, critically, redwings responded to a significantly higher proportion of seet playbacks compared with chip playbacks. Our results suggest that redwing males selectively respond to the seet calls of yellow warbler neighbors as much as they do to nearby cowbirds, and more so than chip calls. For yellow warblers, seet calls warn against brood parasites, while chip calls warn of general predatory threats both to the adult and to the nest (Gill & Sealy, 2003, 1996). Differential redwing responses to the calls suggest that this host species is eavesdropping on and possibly discriminating between the two heterospecific warbler call types. Thus, it may be possible that redwings have a cognitive algorithm to discern between information in yellow warbler seet and chip calls, although this requires further neurofunctional tests.

In the second experiment, we found that when playbacks were presented on redwing territories, both males and females responded to seet calls with similar aggression (in terms of latency, alarm calling and closest approach) to cowbird chatters and blue jay calls, and both less so than to redwing chatters, indicating redwings treat seet calls with the same urgency as calls that simulate threats to breeding success, regardless of sex. Therefore, redwings appear to perceive seet calls as an indicator of a nest threat present, but not necessarily as a referential alarm call specifically informing brood parasitism threat, because responses to seets and Blue Jay calls did not differ. In turn, redwing females, unlike yellow warblers, appear to lack the nest approach and sitting response when hearing cowbird chatters. The aggressive responses we observed in response to seet calls are likely a generic nest-protection response by redwings, as recorded by our observations. Redwings also responded to redwing chatter with the most aggression, suggesting that they perceive conspecific intruders as the gravest threat among our simulated set of danger cues.

In the third analysis, we found that male vocal aggression toward cowbird and seet calls was negatively correlated with distance from nearest yellow warbler territory. This pattern, however, was not seen in females. These results suggest a “neighborhood watch” effect, in that redwing males nesting closer to yellow warblers may be more sensitive to the latter species’ referential seet calls (and the presence of cowbirds through their calls). As such, red wings who have more access to the seet warning calls mount stronger frontline defenses to cowbirds and the seets compared with redwings that have access to this information. In addition, there was no difference between male redwings’ own alarm responses to playbacks of seets vs. cowbird chatters, suggesting that redwings closer to yellow warblers are more responsive to both the warblers’ referential calls and the cowbirds’ own vocalizations that they reference. Greater redwing mobbing through stronger responses to yellow warbler anti-parasitic alarm calls may also contribute to the observed higher nesting success of yellow warblers nearer to redwing nests (Clark & Robertson, 1979). Redwings frontload their nest defenses against brood parasitism Gill et al., 2008; Robertson & Norman 1976, 1977; Ortega & Cruz, 1988; Neudorf & Sealy, 1992, Gill et al., 1997a; Strausberger & Horning, 1997; Cruz, 1999; Yasukawa et al., 2016), so the ability to respond specifically to anti-parasitic seet calls as a warning for cowbirds could impart fitness benefits to redwings that eavesdrop on the call. This, along with the often close nesting proximity between redwing and yellow warbler nests at wetland sites (Clark & Robertson, 1979; Lowther et al., 1999; Yasukawa & Searcy, 2019, pers. obs.), may have primed redwings evolutionarily to pay attention to the warbler seet call.

There are other wetland species that may potentially eavesdrop on seet calls, but we do not see evidence of this in our data. Specifically, when we examined the responses of species other than yellow warblers and redwings during these same set of playbacks at yellow warbler territories (see Supplementary Table 6), we found an opposite pattern, whereby other locally breeding species responded more to the yellow warbler's chip playbacks (20/31 trials, 12 species combined) than to seet calls (8/34 trials, 7 species combined) (Fisher's exact test, $p < 0.05$). Furthermore, while some of the species responded several times to different chip trials, only one species ever appeared in more than one seet trial. Hence, eavesdropping on heterospecific seet calls as a warning signal may be an attribute unique to redwings at our study sites.

From our data, it appears redwings actively eavesdrop on the seet calls of nearby yellow warblers and respond aggressively when exposed to seet calls, both on their own territories as well as neighboring yellow warbler territories. However, we found general behavioral equivalency in the strengths of the responses not only to seet calls and cowbird chatters, but also to blue jay calls (Experiment 2, Figure 4), suggesting that redwings may perceive seets as a general alarm call rather than a referential alarm call for cowbird. Nonetheless, on yellow warbler territories redwings did respond more to cowbird and seet playbacks compared chip playbacks, suggesting there may be some discrimination between heterospecific general alarm calls and anti-parasitic referential calls, but this needs further testing.

A limitation of our study is that data were only collected during incubation, when the risk of brood parasitism is highest. In the future, conducting playbacks across different nesting stages may help us better understand whether redwings can respond to the referential meaning of the yellow warbler's seet call. Prior work (Neudorf & Sealy, 1992) demonstrated that during incubation stage, redwings respond more to cowbird models than models of a nest predator. After the eggs hatch and brood parasitism is no longer a threat, responses to cowbird models decrease, while aggression toward nest predator models increase with increasing investment in young. If redwings are using seet calls as frontline nest defense specifically against brood parasitism, we would expect aggressive responses to seet calls to be strongest during laying and incubation and weaker after the eggs hatch.

In conclusion, our results demonstrate that redwings actively eavesdrop on and respond to yellow warbler seets as a frontline defense to protect their nest investment. Redwings do not appear to have a referential system of their own and may instead eavesdrop on the yellow warbler’s seet call to use it for their benefit. In addition, redwings that nest near yellow warblers respond more strongly to parasitic cues. Given that redwing proximity has been shown to reduce the probability of brood parasitism by cowbirds upon yellow warbler nests nearby (Clark & Robertson, 1979), our own findings open up questions for future research to explore whether yellow warblers and redwings possibly have a mutualistic communicative relationship, whereby yellow warblers are the alarm system providing warning cues for cowbird presence, and redwings are the aggressors keeping cowbirds at bay.

# CHAPTER 6: EAVESDROPPING ON REFERENTIAL YELLOW WARBLER ALARM CALLS BY RED-WINGED BLACKBIRDS IS MEDIATED BY BROOD PARASITISM RISK

## 6.1 ABSTRACT

Referential alarm calls that denote specific types of dangers are common across diverse vertebrate lineages. Different alarm calls can indicate a variety of threats, which often require specific actions to evade. Thus, to benefit from the call, listeners of referential alarm calls must be able to decode the signaled threat and respond to it in an appropriate manner. Yellow warblers (*Setophaga petechia*) produce referential "seet" calls that signal to conspecifics the presence of nearby obligate brood parasitic brown-headed cowbirds (*Molothrus ater*), which lay their eggs in the nests of other species, including yellow warblers. Our previous playback experiments have found that red-winged blackbirds (*Agelaius phoeniceus*), a species also parasitized by brown-headed cowbirds, eavesdrop upon and respond strongly to yellow warbler seet calls during the incubation stage of breeding with aggression similar to responses to both cowbird chatters and predator calls. To assess whether red-winged blackbird responses to seet calls vary with their own risk of brood parasitism, we presented the same playbacks during the nestling stage of breeding (when the risk of brood parasitism is lower than during incubation). As predicted, we found that blackbirds mediated their aggression toward both cowbird chatter calls and the warblers' anti-parasitic referential alarm calls in parallel with the low current risk of brood parasitism during the nestling stage. These results further support that red-winged blackbirds flexibly respond to yellow warbler antiparasitic referential calls as a frontline defense against brood parasitism at their own nests.

## 6.2 INTRODUCTION

Acoustic signals are used across diverse lineages to signal a variety of information, such as food sources or predatory threats (Bradbury and Vehrencamp, 2011). Some acoustic signals, known as functionally referential calls, denote to specific objects in the environment, and are often used to warn conspecifics of particular predator types (e.g., flying vs. ground), each requiring different behavioral responses to evade (Evans et al., 1993; Oda and Masataka, 1996; Evans, 1997; Rainey et al., 2004a, b; Zuberbühler, 2009; Suzuki, 2012). Listeners to referential calls must therefore be able to understand what is being referenced to determine the appropriate response based on the risk posed to them by the specific predatory threat denoted. Heterospecific eavesdropping upon referential calls is common across birds and mammals (Sherman, 1977; Magrath et al., 2015). Heterospecific eavesdroppers often demonstrate the same abilities as intended conspecific receivers do in decoding and responding appropriately to the information contained within the referential signals (e.g., Oda and Masataka, 1996; Rainey et al., 2004b; Suzuki, 2012; see Magrath et al., 2015, 2020 for reviews). For example, Verreaux's sifakas (*Propithecus verreauxi*) produce different referential alarm calls for aerial vs. terrestrial predators, which are heard by both intended conspecific receivers and by eavesdropping heterospecific black-casqued hornbills (*Ceratogymna atrata*) (Rainey et al., 2004a,b). Both types of listeners react to aerial alarm calls by hiding under cover, but, critically, the hornbills do not respond to the alarm calls signaling ground predators because they do not pose a threat to these birds.

Conspecific and heterospecific eavesdropping upon referential alarm calls occurs in songbirds within the context of improving nest defense or minimizing nest detection by predators (Gill and Sealy, 2003, 2004; Platzen and Magrath, 2005; Davies et al., 2006; Haff and Magrath, 2012; Suzuki, 2015; Yu et al., 2017). Avian nests can be threatened by at least two types of dangers: (1) nest predators that depredate eggs and nestlings, and (2) obligate brood parasites that solely lay their eggs in other species' nests (i.e., hosts), leaving the hosts to care for the costly brood parasitic young (Davies, 2010). Many host species exhibit strong frontline defenses against both threat types, responding aggressively toward both predatory and parasitic intruders on the territories to prevent their direct access to the nest (Welbergen and Davies, 2009; Kilner and Langmore, 2011; Feeney et al., 2012; Feeney and Langmore, 2015). There is some overlap between these nest threats, in that brood parasites may depredate eggs (e.g., mafia hypothesis, farming; Hauber, 2014; reviewed in Soler et al., 2017), and nest predators may also threaten adult survival (e.g., genus Accipiter hawks; Winkler et al., 2020). The main distinction between threats is that nest predators are of risk to hosts throughout the nesting cycle (laying, incubation, and nestling stages), whereas brood parasites pose the gravest risk when nests have eggs. Hosts are generally aggressive toward brood parasites during laying and incubation, when the nest is at highest risk of successful brood parasitism, and less aggressive (compared to other threats such as nest predators) during either the pre-nesting or the nestling stages when the risk of parasitism is low (Neudorf and Sealy, 1992; Gill and Sealy, 1996; Fasanella and Fernández, 2009; Lawson et al., 2021a; see Lawson et al., 2021b for a meta-analysis). Conversely, nest predation costs remain high (even increasing) as the brood ages due to its unchanging outcome (i.e., partial or total reproductive failure; Gill and Sealy, 1996; Fasanella and Fernández, 2009; Ruiz et al., 2018).

There is an adaptive benefit for hosts facing both nest threats to be able to discriminate brood parasites from nest predators and respond based on current risk. For potential hosts, anti-parasitic defense hinges on the early detection of brood parasites prior to the parasites' discovery of the host nests (Sealy et al., 1998). Thus, hosts of brood parasites should evolve to eavesdrop upon referential alarm calls that signal brood parasitism risk as an early warning system to maximize their frontline nest defenses. Yellow warblers (*Setophaga petechia*; hereafter "warblers") emit a referential alarm call to signal the presence of a generalist obligate brood-parasite, the brown-headed cowbird (*Molothrus ater*; hereafter: cowbird) (Gill and Sealy, 2004). Specifically, warblers produce "seet calls" to warn conspecifics of nearby cowbirds. After hearing seet calls or producing them, female warblers return to and sit upon their nest, which may prevent the cowbird from inspecting or laying an egg into the nest (Gill et al., 2008; Lawson et al., 2021c). Seet calls are primarily produced in response to the sight and/or sound of cowbirds themselves or seet calls emitted by conspecific warblers, and almost exclusively during laying and incubation stages, when the nest is at the highest risk of parasitism, and not during the pre-nesting or nestling stages (Sealy et al., 1998; Gill and Sealy, 2004; Gill et al., 2008; Lawson et al., 2021a).

Our previous research found evidence that red-winged blackbirds (*Agelaius phoeniceus*, hereafter "blackbirds"), another North American host of brown-headed cowbirds (Searcy and Yasukawa, 1995; Strausberger, 2001; Shaffer and Goldade, 2003), eavesdrop upon and respond to nearby yellow warbler seet calls during their own laying and incubation stages (Lawson et al., 2020a). Blackbirds are phylogenetically and vocally distinct from yellow warblers, but often nest within the same wetlands as the warblers, with greater proximity to blackbirds linked to lower parasitism upon nearby yellow warbler nests (Clark and Robertson, 1979). Blackbirds are larger than cowbirds and yellow warblers, and frontload their anti-parasitic nest defenses, using both vocal and physical aggression toward cowbirds to prevent them from accessing and parasitizing the nest (Robertson and Norman, 1976, 1977; Ortega and Cruz, 1988; Neudorf and Sealy, 1992; Gill et al., 1997a, 2008; Strausberger and Horning, 1997; Cruz, 1999; Yasukawa et al., 2016). Blackbirds are not known to have a referential alarm call system of their own, but they do eavesdrop upon the seet calls of yellow warbler neighbors: in Lawson et al. (2020a) we found that during the incubation stage, blackbirds of both sexes responded more often to the warblers' seet calls relative to their generic "chip" alarm calls, and with similar urgency and vocal aggression toward playbacks of seet calls as to both cowbird chatters and nest predator calls. However, because there was equal response to both types of threats (brood parasite and nest predator), these findings implied that blackbirds do not perceive seet calls as a cowbird-specific referential signal per se, but rather as an alarm call for a nest threat.

Understanding how referential alarm calls are perceived by heterospecifics can be informed by testing under different conditions, such as varying levels of risk posed by the referent. For example, yellow warblers themselves respond less aggressively to referential seet calls during the nestling stage likely because there is little to no brood parasitism risk during this stage (Neudorf and Sealy, 1992; Gill and Sealy, 1996), and the same pattern can be seen across other hosts toward models of their respective brood parasites (Fasanella and Fernández, 2009; reviewed in Lawson et al., 2021b). Furthermore, blackbirds presented with taxidermy cowbird and nest predator models across nesting stages respond equally to both models during incubation, but more strongly to the nest predator during nestling stage (Neudorf and Sealy, 1992; also see Henger and Hauber, 2014). To determine whether blackbirds recognize seet calls as referential alarm calls denoting brood parasitism risk, we expanded on our previous playback study conducted during the incubation stage (Lawson et al., 2020a), and presented playbacks of cowbird chatters, seet calls, nest predator calls, and a non-threatening control species to blackbird nests during the nestling stage, when the risk of brood parasitism is low. We predicted that if blackbirds respond to seet calls as a referent for brood parasites, aggressive responses toward cowbird chatter and seet calls should be lower than aggressive responses to nest predator calls, but comparable to each other, during the later stage of nesting.

## 6.3 MATERIALS AND METHODS

This playback experiment was conducted during April–July 2020 and used the same sites, playback files, and playback methodology as in Lawson et al. (2020a), which tested blackbirds' responses to the same playbacks during the egg/incubation stages during the prior 2 years. The methodology is described briefly below; for more detailed methodology, see Lawson et al. (2020a).

### 6.3.1 Sites and study species

Playbacks occurred at sites in Champaign (n = 3) and Vermilion counties (n = 3) in east central Illinois, United States, where blackbirds and yellow warblers both breed (Lawson et al., 2020a). Both species are parasitized by cowbirds in Illinois (Rodewald, 2015; Merrill et al., 2017; pers. obs.). Blackbirds arrive as early as February but do not breed until late-April through late-July, with peak breeding season mid-May to mid-June (Lawson et al., 2020a; Yasukawa and Searcy, 2020). Yellow warblers arrive on the breeding grounds in late-April with peak breeding mid-to-late May (overlapping with blackbirds; Kelly et al., 2019; Lawson et al., 2021a,c).

### 6.3.2 Playback stimuli construction

For our experiments, we used four of the playback treatments from Lawson et al. (2020a): (1) female cowbird chatter (brood parasite), (2) yellow warbler seet calls [cowbird-specific anti-parasitic alarm call (Gill et al., 1997a; Sealy et al., 1998; Gill and Sealy, 2003, 2004; Gill and Bierema, 2013), (3) blue jay (*Cyanocitta cristata*, a nest predator commonly seen at our sites; Smith et al., 2013), calls and (4) wood thrush (*Hylocichla mustelina*, a non-threatening sympatric heterospecific control, Kelly et al., 2019) songs. Including a nest predator call along with a brood parasite was critical to determine whether the blackbirds' responses to the seet call are antiparasitic or general (Rothstein and Robinson, 1998). Audio file construction is described in detail in Lawson et al. (2020a). Briefly, audio files were edited and filtered in Adobe Audition CC 2019 and included five exemplar files for each treatment sourced from different individuals, with one exemplar chosen randomly for each playback trial to avoid pseudoreplication (Kroodsma et al., 2001). Each exemplar contained vocalizations from at least three individuals.

### 6.3.3 Playback experiment

We conducted playback trials at active blackbird nests that were ≥50 m apart, which is the mean territory size for blackbirds (Searcy and Yasukawa, 1995). Blackbirds are polygynous harem breeders, and are highly defensive of territory boundaries with little to no overlap with other males, with females being site-faithful to male territories (Searcy and Yasukawa, 1995). Therefore, only testing nests ≥50 m apart (i) reduced the likelihood that we tested the same parents twice at different nests, as the subjects were not banded, and (ii) allowed us to accurately record the stages of any additional nests on the male's territory. We searched sites 1–2 times weekly for active nests. Nest contents were checked every 3 days to ensure playback trials occurred during the nestling stage. We conducted playbacks at nests that only contained nestlings <9 days old to prevent forced fledging when inspecting the nest (blackbirds naturally fledge at 11–14 days old; Yasukawa and Searcy, 2020). Playbacks were conducted between 05:00 and 12:00 h local time with a FOXPRO NX4 game caller, placed ∼5 m from active nests. We placed the caller ∼1 m high in vegetation when possible and recorded data from >10 m away. Playback trials occurred for 10 min and were adjusted to broadcast at ∼90 dB at 1 m from the source (Lawson et al., 2020a).

Blackbird nests received two of the four playback treatments, each on a separate day: cowbird chatter (n = 23), yellow warbler seet calls (n = 22), blue jay calls (n = 20), and wood thrush songs (n = 17), for a total of 82 playbacks. The time lapse in between the first and second playback at each territory ranged from 24 to 72 h later (mean = 30.4 h) to avoid habituation. Nests were randomly assigned treatments to minimize the potential for an effect of treatment order. Six nests were not retested as they were depredated between trials. Furthermore, the focal female did not appear within the playback range for two of the trials, and thus, these trials were dropped from the data analyses.

During the playback trial we recorded the following behavioral responses from both parents within 30 m of the speaker: (1) response latency (sec after the start of trial when a switch to behaviors signaling playback detection occurred: posturing, hopping, alarm calling, or attacking the speaker) (2) closest approach to the speaker (m); and (3) the number of alarm calls produced ("checks," "chits," "chonks" used interchangeably as nest defense alarm calls by both sexes, and "cheers" which are only produced by males, Beletsky et al., 1986; Knight and Temple, 1988; Yasukawa, 1989). We only recorded responses of the focal male and focal nesting female (determined by observing which female fed the nestlings), and not other females within the harem. The focal birds were visually tracked by an observer throughout the entire trial while another recorded the behaviors. The presence of additional nest(s) with eggs, as well as age of nestlings in the focal nest, were included as variables in our models (see section "Statistical Analyses").

These studies were approved by the Animal Ethics Committee (IACUC) of the University of Illinois (#17259), and by United States federal (MB08861A-3) and Illinois state agencies (W20.6394).

### 6.3.4 Statistical analyses

We evaluated whether playback treatment affected the same three response variables of interest (latency, total alarm calls, and closest approach) using a separate generalized linear model for each. Models were also separated by sex, due to the polygynous nature of blackbirds possibly leading to sex differences in nest defensive behaviors (Yasukawa and Searcy, 2020). For all latency and alarm call models we used a negative binomial general linear model to account for the large number of non-responses (0 s latency, no alarm calls produced) that varied by treatment. For the closest approach variable, we log-transformed the data after adding a small constant to obtain a normal distribution, and ran a linear model. All models included the following fixed effects: playback treatment, date (ordinal days after start of season – April 1st), trial order (to account for repeated playbacks at the same site), and age of nestlings (to account for the variation in ages of nests at time of playback). For models on male data, we also included the presence of another nest with eggs as another fixed effect, because males may have multiple females at different stages of nesting on their territory, and if there were differences in response over nest stages, presence of eggs may have affected the males' responses. If the presence of eggs was significant, we ran the same generalized linear model with an interaction term (treatment × presence of eggs) to determine if responses to specific treatments were affected by presence of a nest with eggs on the male's territory. For all models with significance, we ran post hoc Tukey tests to multiple compare treatment pairs of least-square means. All statistical tests were conducted in the statistical program R 4.0.5 (packages lme4, nlme, multcomp, emmeans, and car), with $\alpha = 0.05$. Effect sizes were calculated in R for all significant and non-significant outcomes.

## 6.4 RESULTS

### 6.4.1 Latency

Average latencies to respond varied significantly by treatment for both males ($F_{3,81} = 8.95$, $p < 0.001$; Figure 28) and females ($F_{3,79} = 7.02$, $p < 0.001$; Figure 29). Based on post hoc pairwise comparisons of least-square means, males responded more quickly to playbacks of blue jay calls compared to cowbird chatters ($z = 4.44$, $p < 0.001$), seet calls ($z = 6.30$, $p < 0.001$), and control wood thrush songs ($z = 6.25$, $p < 0.001$). Female latencies showed the same pattern, where females responded more quickly to playbacks of blue jay calls compared to cowbird chatters ($z = 3.14$, $p < 0.01$), seet calls ($z = 3.06$, $p = 0.01$), and control wood thrush songs ($z = 2.92$, $p = 0.01$). There was no significant difference in latency to respond to cowbird chatters compared to seet calls for either sex (males: $z = -1.80$, $p = 0.27$; females: $z = 1.13$, $p = 0.99$), and both sexes responded to cowbird and seet calls with similar latency to the control wood thrush (males: cowbird-wood thrush $z = -2.02$, $p = 0.18$, seet-wood thrush $z = -3.99$, $p = 0.97$; females: cowbird-wood thrush $z = 0.005$, $p = 0.99$, seet-wood thrush $z = -0.11$, $p = 0.99$; see Supplementary Table 10 for all post hoc comparisons). For both sexes, neither date of playback (males: $F3,81 = 0.37$, $p = 0.54$, estimate $= -0.009$; females: $F3,79 = 1.10$, $p = 0.29$, estimate $<-0.001$) nor trial order (males: $F3,81 = 0.33$, $p = 0.56$, estimate $= 0.26$; females: $F3,79 < 0.01$, $p = 0.93$, estimate $= -0.01$) affected latency responses. While age of nestlings did not significantly influence male latency ($F3,81 = 3.07$, $p = 0.08$, estimate $= 0.22$), females responded more quickly to playbacks with increasing age of nestlings ($F3,79 = 3.93$, $p = 0.05$, estimate $= 0.12$). For males, presence of a nest with eggs on the territory did not significantly affect latency responses ($F3,81 = 3.15$, $p = 0.07$, estimate $= -1.4$).

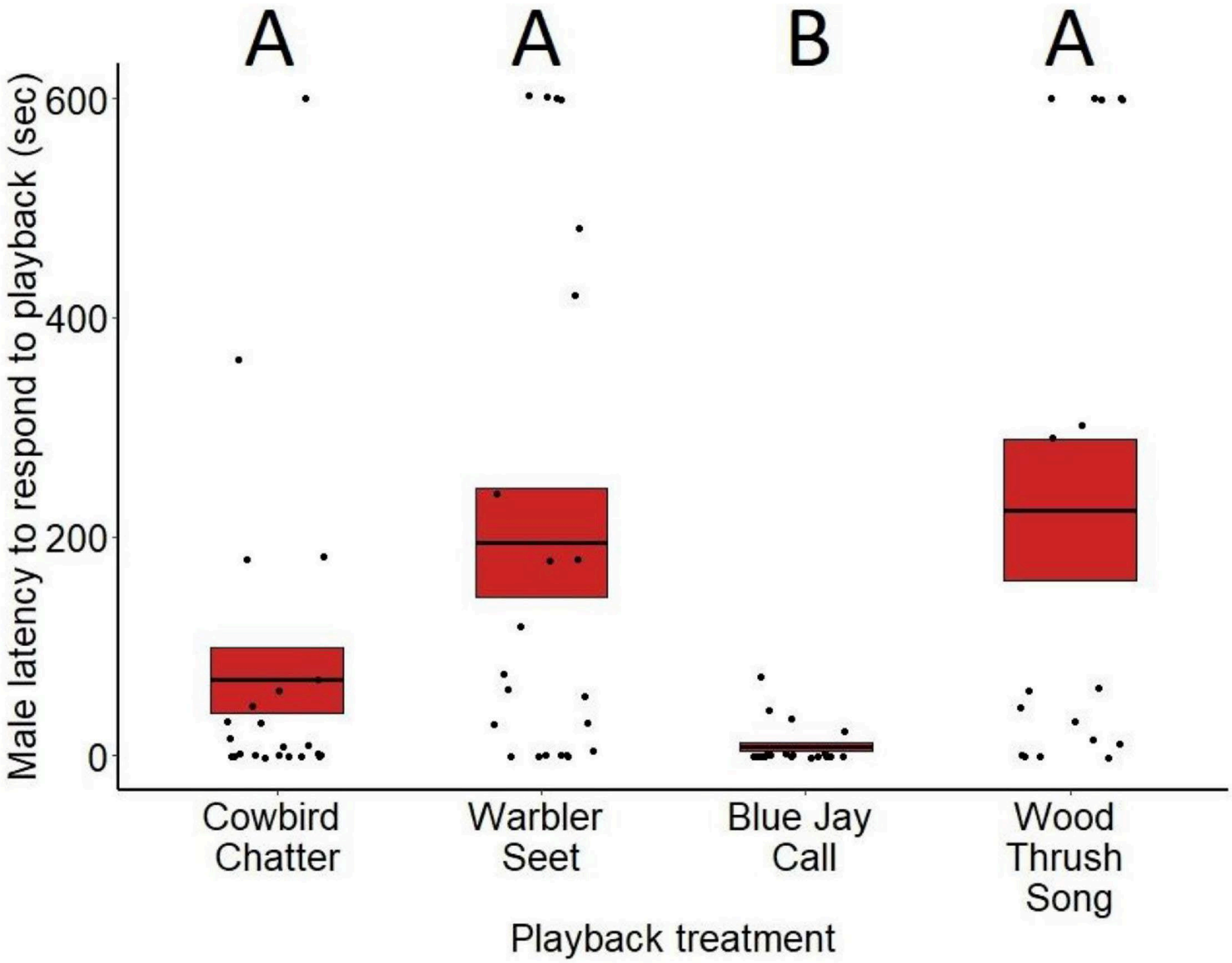

**Figure 28.** Latency (in seconds) for male red-winged blackbirds to respond to the playback treatments at nests during nestling stage. Means are shown with the bold line, and shaded boxes represent standard errors. Boxes with different letters denote post hoc statistical differences between treatments. For the p-values of post hoc comparisons, please refer to Supplementary Table 10.

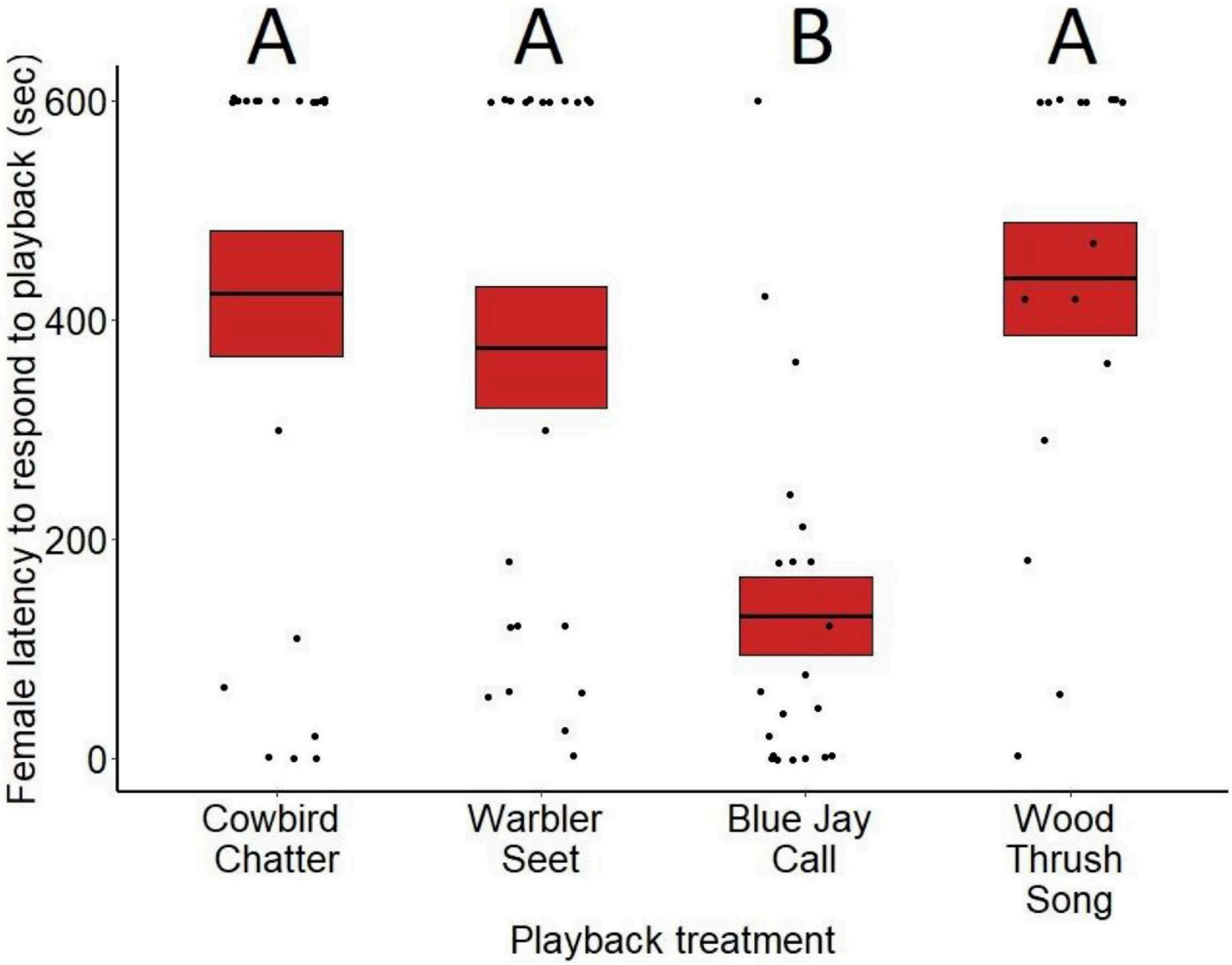

**Figure 29.** Latency (in seconds) for female red-winged blackbirds to respond to the playback treatments at nests during nestling stage. Means are shown with the bold line, and shaded boxes represent standard errors. Boxes with different letters denote post hoc statistical differences between treatments. For the p-values of post hoc comparisons, please refer to Supplementary Table 10.

### 6.4.2 Closest approach

Closest approach varied significantly by treatment for males ($F_{3,81} = 5.55$, $p < 0.01$; Figure 30) but not females ($F_{3,79} = 1.18$, $p = 0.32$; Figure 31). Based on post hoc comparisons, males approached playbacks of cowbird chatters more closely than playbacks of seet calls ($z = 2.73$, $p = 0.03$) and control wood thrush songs ($z = 3.81$, $p < 0.001$). Males also approached blue jay calls more closely than wood thrush songs ($z = 3.20$, $p = 0.05$). Closest approach did not differ between any of the other playback comparisons (cowbird-blue jay $z = -1.33$, $p = 0.54$, blue jay-seet $z = -1.34$, $p = 0.53$, seet-wood thrush z = −1.35, p = 0.52; see Supplementary Table 11 for all post hoc comparisons). For both sexes, neither date of playback (males: F3,81 = 0.30, p = 0.58, estimate = 0.004; females: F3,79 < 0.001, p = 0.98, estimate <−0.001), trial order (males: F3,81 = 0.40, p = 0.52, estimate = −0.15; females: F3,79 < 0.68, p = 0.40, estimate = −0.16), nor age of nestlings (males: F3,81 = 0.17, p = 0.68, estimate = −0.02; females: F3,79 = 0.87, p = 0.98, estimate = 0.04) affected closest approach. For males, presence of a nest with eggs on the territory also did not significantly affect closest approach (F1,81 = 3.20, p = 0.07, estimate = −0.60).

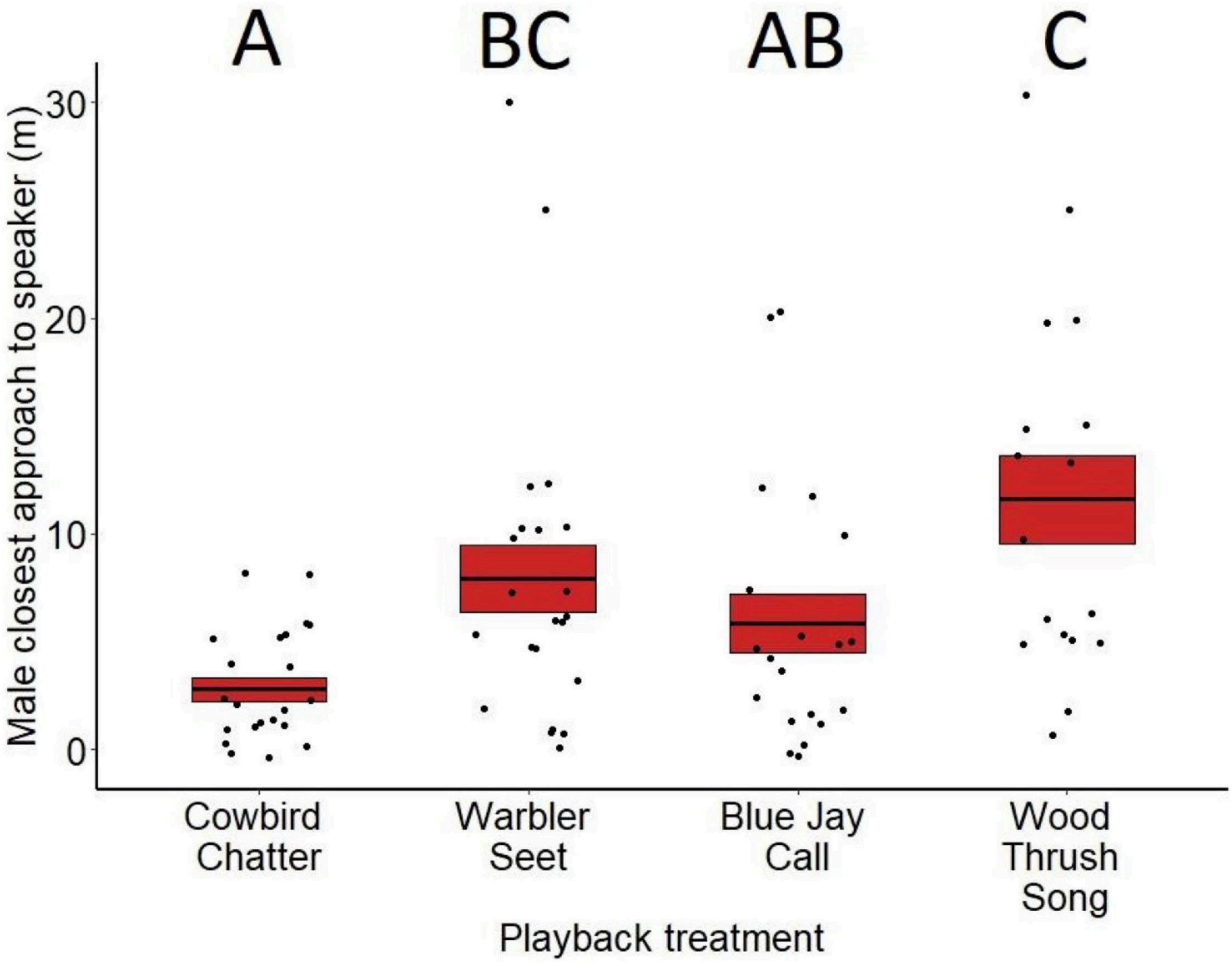

**Figure 30.** Closest approach to the playback speaker (in meters) by male red-winged blackbirds for the different treatments at nests during nestling stage. Means are shown with the bold line, and shaded boxes represent standard errors. Boxes with different letters denote post hoc statistical

**Figure 30. (Cont.)** differences between treatments. For the p-values of post hoc comparisons, please refer to Supplementary Table 11.

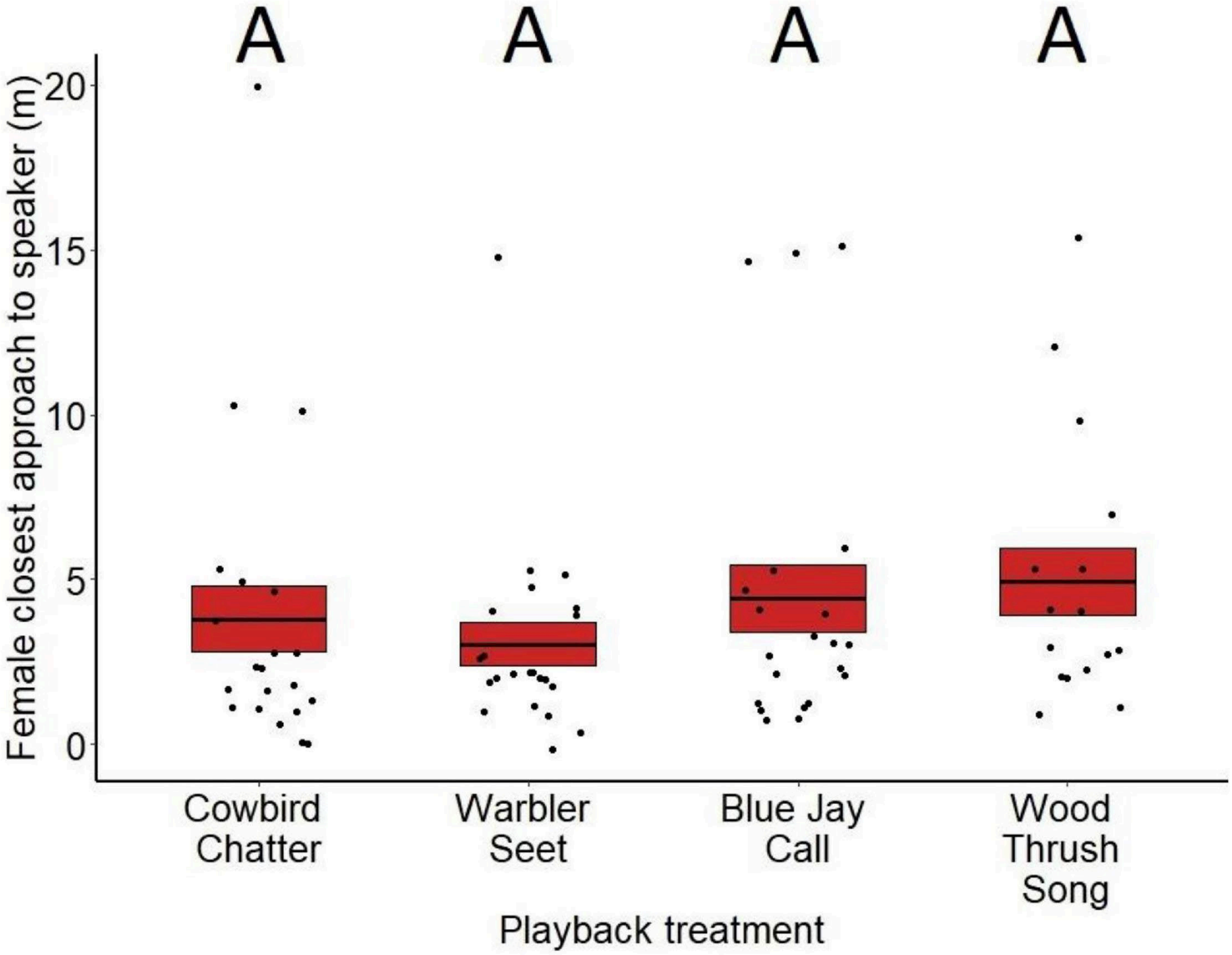

**Figure 31.** Closest approach to the playback speaker (in meters) by female red-winged blackbirds for the different treatments at nests during nestling stage. Means are shown with the bold line, and shaded boxes represent standard errors. There were no significant pairwise differences; for the p-values of post hoc comparisons, please refer to Supplementary Table 11.

### 6.4.3 Alarm calling

Alarm calling varied significantly between treatments for both males (F3,81 = 6.55, $p < 0.001$; Figure 32) and females (F3,79 = 8.92, $p < 0.001$; Figure 33). Based on post hoc pairwise comparisons of least-squares means, males alarm called more toward playbacks of blue jay calls compared to cowbird chatters ($z = 2.53$, $p = 0.05$), seet calls ($z = 3.31$, $p < 0.01$), and control wood thrush songs (z

$= 3.75$, $p < 0.001$). Female also alarm called more toward blue jay calls compared to cowbird chatters ($z = 5.84$, $p < 0.001$), seet calls ($z = 5.99$, $p < 0.001$), and control wood thrush songs ($z = 3.33$, $p < 0.01$). There was no significant difference in alarm calling responses toward cowbird chatters compared to seet calls for either sex (males: $z = 0.73$, $p = 0.88$; females: $z = 0.05$, $p = 0.99$), and both sexes alarm called similarly toward cowbird and seet calls compared to the control wood thrush (males: cowbird-wood thrush $z = 1.34$, $p = 0.53$, seet-wood thrush $z = 0.69$, $p = 0.90$; females: cowbird-wood thrush $z = -2.11$, $p = 0.14$, seet-wood thrush $z = 2.16$, $p = 0.13$; see Supplementary Table 12 for all post hoc comparisons). For both sexes, date of playback had a significant effect on alarm calling (males: $F3,81 = 4.59$, $p = 0.03$, estimate $= 0.01$; females: $F3,79 = 8.28$, $p < 0.01$, estimate $= 0.03$), with blackbirds producing more alarm calls later in the season. Females also alarm called significantly more with increasing age of the nestlings in her nest ($F3,79 = 4.06$, $p = 0.04$, estimate $= -0.22$), and while males showed this same pattern, it was non-significant ($F3,81 = 3.65$, $p = 0.06$, estimate $= 0.12$). Trial order did not significantly affect alarm call responses (males: $F3,81 = 1.27$, $p = 0.26$, estimate $= -0.27$; females: $F3,79 = 2.56$, $p = 0.11$, estimate $= -0.75$).

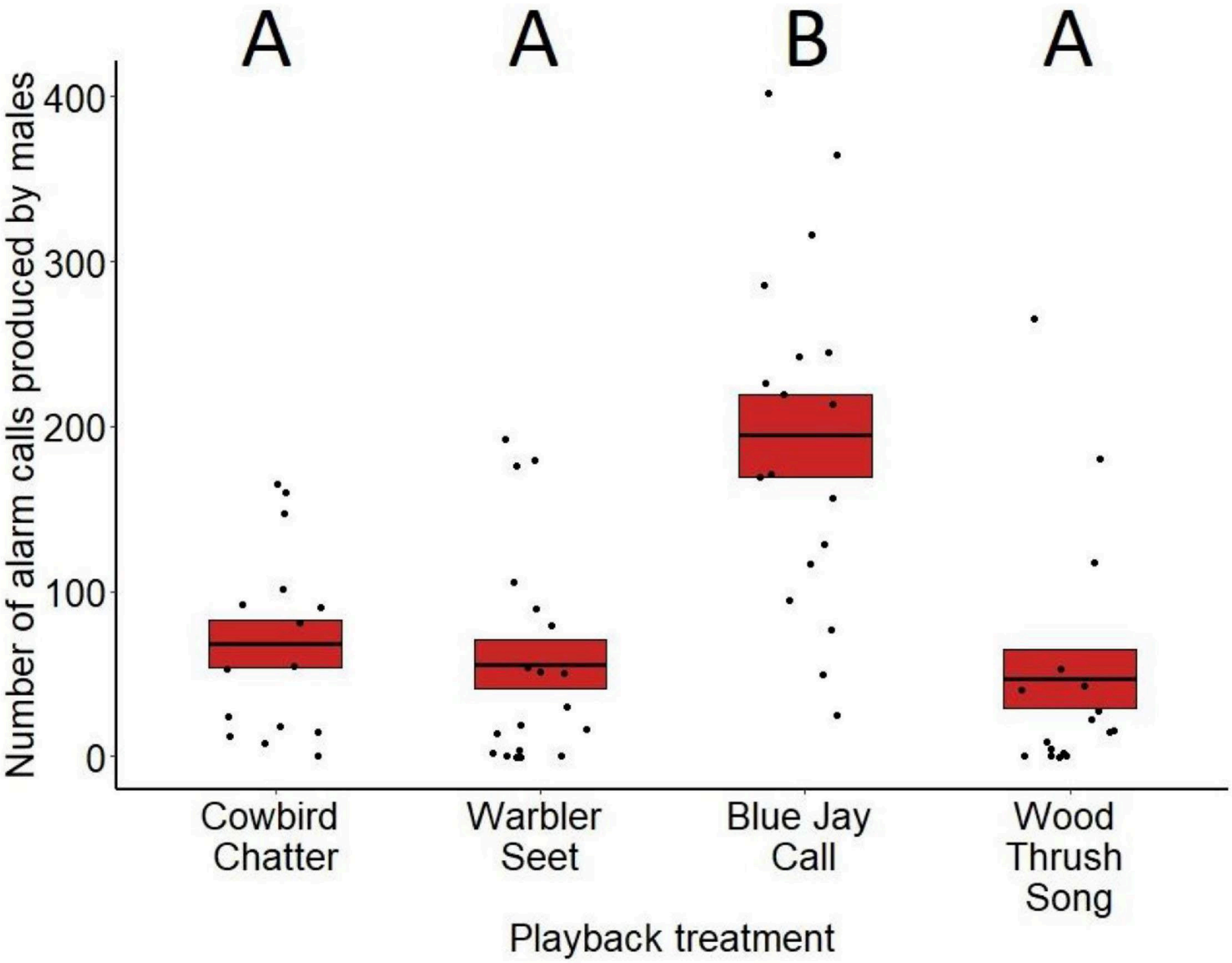

**Figure 32.** Number of alarm calls produced by male red-winged blackbirds in response to playbacks at nests during nestling stage. Trials where males had an additional nest on territory with eggs are excluded. Means are shown with the bold line, and shaded boxes represent standard errors. Boxes with different letters denote post hoc statistical differences between treatments. For the p-values of post hoc comparisons, please refer to Supplementary Table 12.

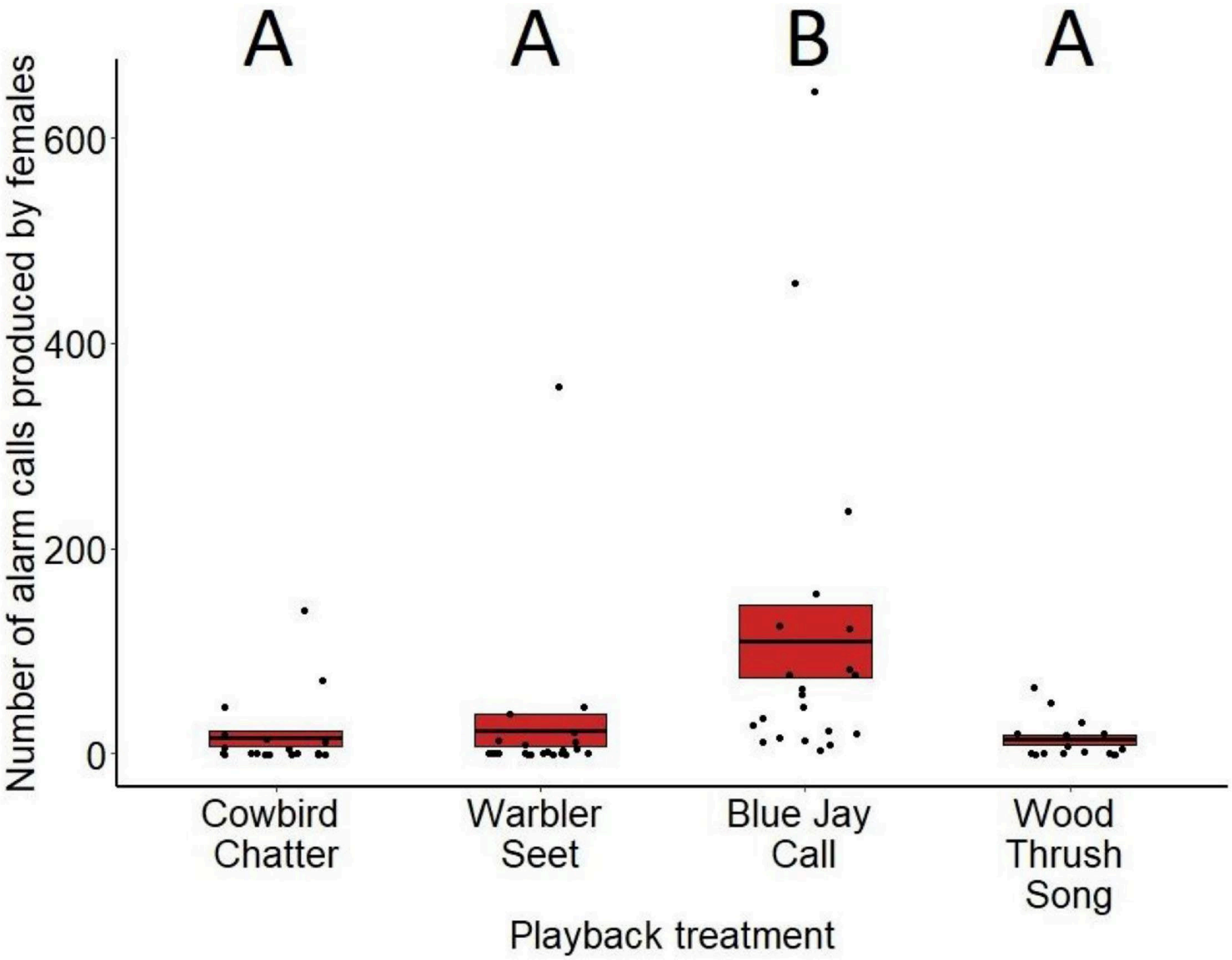

**Figure 33.** Number of alarm calls produced by female red-winged blackbirds in response to playbacks at nests during nestling stage. Means are shown with the bold line, and shaded boxes represent standard errors. Boxes with different letters denote post hoc statistical differences between treatments. For the p-values of post hoc comparisons, please refer to Supplementary Table 12.

For males, the presence of an additional nest with eggs on their territory significantly affected alarm calling responses ($F_{3,81} = 8.25$, $p < 0.01$, estimate = 0.95). When we ran the same generalized linear model with an interaction term we found a significant interaction between treatment and presence of a nest with eggs on alarm calling rates (treatment × eggs term: $F_{3,64} = 5.01$, $p < 0.01$). Specifically, males alarm called more toward playbacks of cowbird chatters (z ratio = 2.60, $p < 0.01$) and seet calls (z ratio = 2.63, $p < 0.01$) when there was an additional nest with eggs on their territory, while alarm calling toward blue jay playbacks was not significantly affected by presence of eggs vs. nestlings only (z ratio = −1.72, $p = 0.08$) (wood thrush playbacks were not included since no territories with the wood thrush playback had a blackbird nest with eggs). To determine how alarm calling between treatments varied without the influence of nests with eggs, we removed any trials with territories with eggs and reran the original general linear model. Male alarm calling still varied significantly by treatment (F3,81 = 6.57, $p < 0.001$), with post hoc comparisons showing the same patterns of higher alarm calling toward playbacks of blue jay calls compared to cowbird chatters ($z = 2.64$, $p = 0.04$), seet calls ($z = 3.40$, $p < 0.01$), and control wood thrush songs ($z = 3.71$, $p = 0.001$) as the model with no eggs. In addition, males showed no significant differences in responses between cowbird chatters and seet calls ($z = 0.52$, $p = 0.95$), between cowbird chatters and the control ($z = 0.93$, $p = 0.78$), and between seet calls and the control ($z = 0.43$, $p = 0.97$), similar to males with nestlings only.

## 6.5 DISCUSSION

Our current playback study found support for the hypothesis that both male and female red-winged blackbirds eavesdrop upon and respond to yellow warbler's seet calls specifically as a referent for "brood parasite" and not as a general nest-threat heterospecific alarm call. On the one hand, the risk of brood parasitism for hosts is highest when nests have eggs, and lower after the eggs hatch; on the other hand, the risk of predation remains high and often increases across nest stages, as the fitness outcome generally is the same – partial or total reproductive failure (Gill and Sealy, 1996; Fasanella and Fernández, 2009; Ruiz et al., 2018). In our own work, blackbirds demonstrated markedly different patterns of response toward brood parasitic vs. predatory threat playbacks depending on the risk posed by them across nest stages. During the nestling stage in the current study, blackbirds of both sexes responded equally and with low aggression toward calls signaling brood parasites (cowbird and seet calls) as to the control wood thrush songs, responding instead most aggressively toward nest-predatory blue jay calls. Contrastingly, when the same playbacks were presented during the incubation stage, blackbirds responded with equally strong aggression toward playbacks of cowbird chatters, seet calls, and blue jay calls (Lawson et al., 2020a). Thus, blackbirds mediated in parallel their urgency to respond and aggression toward calls signaling brood parasitic danger, including referential seet calls of yellow warblers, depending on the level of the current threat of parasitism to their nest(s). Blackbirds mediated aggression depending on date in season and the age of their nestlings as well, showing increased aggression as the breeding season progressed and with increased age of their nestlings. Costs of renesting increase as the season progresses and reproductive value of offspring due to increased chance of survival increases (Montgomerie and Weatherhead, 1988; Gill and Sealy, 1996; Fasanella and Fernández, 2009; Ruiz et al., 2018) parents are thus expected to increase aggression toward threats toward their offspring with the greater age of their young. Similar patterns have been observed in other presentation studies with alarm-calling species (Regelmann and Curio, 1983; Montgomerie and Weatherhead, 1988; Campobello and Sealy, 2010; Lawson et al., 2021a,b).

A relevant distinction between male and female blackbirds is that in this polygynous mating system (Searcy and Yasukawa, 1995), males may have multiple nests at once on their territory, some even at different stages of development, while females only actively care for one nest. Males do not incubate eggs and provide limited paternal provisions for nestlings (e.g., Li and Hauber, 2021), but rather perform a sentinel role of protecting the territory from threats (Yasukawa and Searcy, 2020). However, our findings suggest that males actively monitor the progress of all nests within their harem, as those with additional nests with eggs still vulnerable to brood parasitism responded more strongly to cowbird chatters and seet calls compared to males with nests solely at the nestling stage. This statistical effect was not seen in blackbirds with eggs responding to blue jay calls, indicating a specifically anti-parasitic nest defense. This corresponds with previous work that showed male blackbirds alter provisioning rates based on age of nestlings, even after nests were swapped, supporting that male blackbirds actively monitor all the nests within their harem (Yasukawa et al., 1993). Blackbird males are also known to pay attention to social and vocal cues of females on their territories (Yasukawa, 1989), and this may also include cues from females regarding brood parasitism risk, leading to adjustments in the male's responses to cowbirds and cowbird-signaling calls.

Personal information vs. social information on risk likely affects host responses, as each has different reliability and cost. For example, we found that males approached cowbird and blue jay calls more closely than seet calls. The pattern of male closest approach was similar to that found by blackbirds during the incubation stage (Lawson et al., 2020a), as well as to male yellow warblers during the incubation stage as well (Lawson et al., 2021a). Though both cowbird chatters and seet calls indicate brood parasitism risk, cowbird chatters directly indicate cowbird presence, whereas seet calls indirectly do so. Yellow warblers and blackbirds alike appear to more closely approach playbacks that directly signal threats (cowbird and blue jay calls) compared to social information of risk (seet calls) as acoustic presentations alone provide no visual target for responding subjects to direct physical aggression toward. Campobello and Sealy (2011a) found similar patterns in responses of yellow warblers presented with personal (cowbird model on nest, nest parasitized) or social information (conspecifics mobbing cowbird) on brood parasitism risk, where warblers responded more strongly to individually learned information. Conversely, reed warblers (*Acrocephalus scirpaceus*) in a similar experiment showed preference for social information on brood parasitism risk by common cuckoos compared to personal information (Campobello and Sealy, 2011b). Therefore, cost of acquiring personal information may also affect reliance on and responses to it, as common cuckoo nestlings eject all host eggs/nestlings from the nest unlike cowbirds (Campobello and Sealy, 2011b). Treatments did not influence female closest approach because females spent most of the time alarm calling near or on the nest during playbacks, resulting in an average of ~5 m approaches across treatments, as this was the distance the speaker was placed from the nest.

Our combined set of blackbird playback studies brings to light new questions in the blackbird-warbler eavesdropping system that should be addressed in future studies. Yellow warblers nesting in close proximity to blackbirds experience lower rates of parasitism (Clark and Robertson, 1979), due to the blackbirds' aggressive frontline defenses toward cowbirds near their territories. Do blackbirds that nest near yellow warblers themselves experience a decrease in brood parasitism rate as well? Blackbirds that nest closer to yellow warblers show increased alarm calling responses to chatters and seet calls (Lawson et al., 2020a), suggesting a "neighborhood watch effect" where blackbirds that have access to the yellow warblers' referential system are more primed to respond to their cowbird-signaling calls. Thus, the relationship between yellow warblers and blackbirds appears mutualistic, yet it is unknown whether blackbirds experience a similar decrease in parasitism of their nests when in proximity to yellow warblers. Our study also encourages future research into how blackbird males and many other host species mechanistically make the switch in behavior toward cowbirds as their nests transition from eggs to chicks. Yellow warblers of both sexes also demonstrate a shift in response toward cowbirds from incubation to nestling stage, but warblers of both sexes also interact with and care for the young, unlike most blackbird males at most nests (Li and Hauber, 2021). The mechanism underlying these shifts in behavior is unknown for either species, although endocrine factors, particularly testosterone and prolactin, play a strong role in parental (including paternal) care and different nest-attentive behaviors across the breeding stages in birds (Wingfield et al., 1990; Schoech et al., 1998; Van Roo et al., 2003; Ketterson et al., 2005; Møller et al., 2005; O'Neal et al., 2008).

Our set of playbacks conducted across nesting stages has led to firm support for heterospecific eavesdropping on a referential call signaling the presence of obligate brood parasites (also see Yu et al., 2019). Blackbirds appear to perceive the seet call as a warning specifically for brood parasitic danger, priming them for defensive responses to actual cowbirds. Moreover, blackbirds respond to warbler seets and cowbird chatters based on current risk of brood parasitism to their nests. Future research is needed to measure parasitism rates and fitness benefits of blackbirds nesting near yellow warblers; our study suggests that red-winged blackbirds may have a communicative and possibly mutualistic relationship with the warblers, whereby warblers provide the early warning system for cowbirds, and blackbirds keep cowbirds away from nearby nests.

Heterospecific eavesdropping on alarm calls signaling threats to fitness are seen across diverse taxa, including networks of co-existing species (e.g., tropical mixed-species bird flocks: Martínez et al., 2021). Eavesdropping in multi-species networks could improve threat detection in many biologically meaningful contexts (see Magrath et al., 2015 for review), including foraging (e.g., Batcheller, 2017), habitat selection (e.g., Mönkkönen and Forsman, 2002), and offspring defense (this study). It still remains to be seen, however, whether the symmetrical (whereby each interacting species recognizes the other's referential alarm call; Walton and Kershenbaum, 2019) or asymmetrical (whereby only one actor recognizes the other's call; this study) systems are more likely to evolve and be maintained by mutualistic selective forces. Both theoretical modeling and more empirical and meta-analytic work may be able to resolve these broader scale questions.

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# APPENDIX A: SUPPLEMENTARY TABLES AND FIGURE

**Supplementary Table 1.** Pair-wise comparisons of effect size data by nesting stage and general threat type (parasite vs. predator). Significant post hoc differences are represented with asterisks (*** $P < 0.001$, ** $P < 0.01$, * $P < 0.05$)

| Comparisons by nesting stage | | | | |
|---|---|---|---|---|
| Threat | Host type | Contrast (stage) | T ratio | *P* value |
| Parasite | Accepter | Egg - Nestling | 1.40 | 0.162 |
| Predator | Accepter | Egg - Nestling | -3.22 | 0.001** |
| Parasite | Rejecter | Egg - Nestling | 0.68 | 0.493 |
| Predator | Rejecter | Egg - Nestling | -1.42 | 0.155 |
| Comparisons by threat type | | | | |
| Nest stage | Host type | Contrast (threat) | T ratio | *P* value |
| Egg | Accepter | Parasite - Predator | 0.76 | 0.443 |
| Nestling | Accepter | Parasite - Predator | -3.38 | < 0.001*** |
| Egg | Rejecter | Parasite - Predator | -2.05 | 0.04* |
| Nestling | Rejecter | Parasite - Predator | -3.47 | < 0.001*** |
| Comparisons by host type | | | | |
| Nest stage | Threat type | Contrast (host) | T ratio | *P* value |
| Egg | Parasite | Accepter - Rejecter | 2.12 | 0.035* |
| Nestling | Parasite | Accepter - Rejecter | 1.13 | 0.258 |
| Egg | Predator | Accepter - Rejecter | -0.49 | 0.621 |
| Nestling | Predator | Accepter - Rejecter | 0.49 | 0.618 |

**Supplementary Table 2.** Pair-wise comparisons of effect size data by nesting stage and specific threat type (evictor vs. non-evictor, adult vs. nest predator). Significant post hoc differences are represented with asterisks (*** P < 0.001, ** P < 0.01, * P < 0.05)

| Comparisons by nesting stage | | | | |
|---|---|---|---|---|
| Threat | Host type | Contrast (stage) | T ratio | *P* value |
| Evictor | Accepter | Egg - Nestling | -0.05 | 0.959 |
| Non-evictor | Accepter | Egg - Nestling | 2.04 | 0.041* |
| Adult predator | Accepter | Egg - Nestling | -3.85 | < 0.001*** |
| Nest predator | Accepter | Egg - Nestling | -1.98 | 0.048* |
| Evictor | Rejecter | Egg - Nestling | NA | NA |
| Non-evictor | Rejecter | Egg - Nestling | 0.23 | 0.817 |
| Adult predator | Rejecter | Egg - Nestling | NA | NA |
| Nest predator | Rejecter | Egg - Nestling | -1.44 | 0.149 |
| Comparisons by threat type | | | | |
| Nest stage | Host type | Contrast (threat) | T ratio | *P* value |
| Egg | Accepter | Adult predator - Nest predator | -0.73 | 0.883 |
| Egg | Accepter | Adult predator - Evictor | -0.13 | 0.999 |
| Egg | Accepter | Adult predator - Non-evictor | -1.23 | 0.603 |
| Egg | Accepter | Nest predator - Evictor | 0.51 | 0.956 |
| Egg | Accepter | Nest predator - Non-evictor | -0.82 | 0.843 |
| Egg | Accepter | Evictor - Non-evictor | -0.97 | 0.763 |
| Egg | Rejecter | Adult predator - Nest predator | 0.95 | 0.777 |
| Egg | Rejecter | Adult predator - Evictor | -0.18 | 0.997 |

**Supplementary Table 2. (Cont.)**

| | | | | |
|---|---|---|---|---|
| Egg | Rejecter | Adult predator - Non-evictor | 1.77 | 0.286 |
| Egg | Rejecter | Nest predator - Evictor | -1.60 | 0.378 |
| Egg | Rejecter | Nest predator - Non-evictor | 2.48 | 0.064 |
| Egg | Rejecter | Evictor - Non-evictor | 2.72 | 0.034* |
| Nestling | Accepter | Adult predator - Nest predator | 3.03 | 0.013* |
| Nestling | Accepter | Adult predator - Evictor | 3.49 | 0.003** |
| Nestling | Accepter | Adult predator - Non-evictor | 1.37 | 0.516 |
| Nestling | Accepter | Nest predator - Evictor | 2.88 | 0.021* |
| Nestling | Accepter | Nest predator - Non-evictor | 0.70 | 0.896 |
| Nestling | Accepter | Evictor - Non-evictor | NA | NA |
| Nestling | Rejecter | Adult predator - Nest predator | NA | NA |
| Nestling | Rejecter | Adult predator - Evictor | NA | NA |
| Nestling | Rejecter | Adult predator - Non-evictor | NA | NA |
| Nestling | Rejecter | Nest predator - Evictor | NA | NA |
| Nestling | Rejecter | Nest predator - Non-evictor | 3.47 | 0.003** |
| Nestling | Rejecter | Evictor - Non-evictor | NA | NA |

**Supplementary Table 2. (Cont.)**

| Comparisons by host type | | | | |
|---|---|---|---|---|
| Nest stage | Threat type | Contrast (host) | T ratio | *P* value |
| Egg | Evictor | Accepter - Rejecter | -1.80 | 0.071 |
| Egg | Non-evictor | Accepter - Rejecter | 3.19 | 0.001** |
| Egg | Adult predator | Accepter - Rejecter | -1.18 | 0.236 |
| Egg | Nest predator | Accepter - Rejecter | -0.06 | 0.948 |
| Nestling | Evictor | Accepter - Rejecter | NA | NA |
| Nestling | Non-evictor | Accepter - Rejecter | 0.74 | 0.455 |
| Nestling | Adult predator | Accepter - Rejecter | NA | NA |
| Nestling | Nest predator | Accepter - Rejecter | 0.06 | 0.950 |

**Supplementary Table 3.** Pair-wise comparisons of latency data by pairing status and treatments: Brown-headed cowbird chatters (BHCO), yellow warbler seet calls (Seet), yellow warbler chip calls (Chip), blue jay calls (BLJA), and wood thrush song (WOTH). Significant *post hoc* differences are represented with asterisks (*** $P < 0.001$, ** $P < 0.01$, * $P < 0.05$)

| Comparisons (binomial model of response vs no response) | | z ratio | *P* value |
|---|---|---|---|
| Treatment *post hoc* - Paired Males | BHCO Paired – BLJA Paired | -0.31 | 0.997 |
| | BHCO Paired – Chip Paired | -0.13 | 0.999 |
| | BHCO Paired – Seet Paired | 0.28 | 0.998 |
| | BHCO Paired – WOTH Paired | 3.75 | 0.001** |
| | BLJA Paired – Chip Paired | -0.42 | 0.993 |
| | BLJA Paired – Seet Paired | -0.02 | 0.999 |
| | BLJA Paired – WOTH Paired | 3.51 | 0.004* |
| | Chip Paired – Seet Paired | 0.39 | 0.994 |
| | Chip Paired – WOTH Paired | 3.65 | 0.002** |
| | Seet Paired – WOTH Paired | 3.51 | 0.004** |
| Treatment *post hoc* - Unpaired Males | BHCO Paired – BLJA Paired | 0.24 | 0.999 |
| | BHCO Paired – Chip Paired | -2.30 | 0.144 |
| | BHCO Paired – Seet Paired | -1.07 | 0.818 |
| | BHCO Paired – WOTH Paired | <0.01 | 0.999 |
| | BLJA Paired – Chip Paired | -2.60 | 0.069 |
| | BLJA Paired – Seet Paired | -1.37 | 0.643 |
| | BLJA Paired – WOTH Paired | <0.01 | 0.999 |
| | Chip Paired – Seet Paired | 1.57 | 0.514 |
| | Chip Paired – WOTH Paired | <0.01 | 0.999 |
| | Seet Paired – WOTH Paired | <0.01 | 0.999 |
| **Comparisons (responses only)** | | **z ratio** | ***P* value** |
| Treatment *post hoc* - Paired Males | BHCO Paired – BLJA Paired | 1.41 | 0.493 |
| | BHCO Paired – Chip Paired | 2.71 | 0.041* |
| | BHCO Paired – Seet Paired | 1.87 | 0.249 |
| | BLJA Paired – Chip Paired | 1.22 | 0.614 |
| | BLJA Paired – Seet Paired | 0.47 | 0.965 |
| | Chip Paired – Seet Paired | -0.71 | 0.889 |
| Treatment *post hoc* - Unpaired Males | BHCO Paired – BLJA Paired | 0.61 | 0.925 |
| | BHCO Paired – Chip Paired | 1.60 | 0.384 |
| | BHCO Paired – Seet Paired | 1.55 | 0.413 |
| | BLJA Paired – Chip Paired | 0.71 | 0.889 |
| | BLJA Paired – Seet Paired | 0.65 | 0.914 |
| | Chip Paired – Seet Paired | 0.03 | 0.999 |

**Supplementary Table 4.** Pair-wise comparisons of seet data by pairing status and treatments: Brown-headed cowbird chatters (BHCO), yellow warbler seet calls (Seet), yellow warbler chip calls (Chip), blue jay calls (BLJA), and wood thrush song (WOTH). Significant *post hoc* differences are represented with asterisks (*** $P < 0.001$, ** $P < 0.01$, * $P < 0.05$)

| Comparisons (binomial model of response vs no response) | | z ratio | *P* value |
|---|---|---|---|
| Treatment *post hoc* - Paired Males | BHCO Paired – BLJA Paired | 2.39 | 0.043* |
| | BHCO Paired – Chip Paired | 2.31 | 0.053* |
| | BHCO Paired – Seet Paired | -0.38 | 0.921 |
| | BHCO Paired – WOTH Paired | 2.40 | 0.042* |
| | Seet Paired – BLJA Paired | 2.53 | 0.030* |
| | Seet Paired – Chip Paired | 2.45 | 0.037* |
| | Seet Paired – WOTH Paired | 2.54 | 0.029* |
| Treatment *post hoc* - Unpaired Males | BHCO Paired – BLJA Paired | 0.10 | 0.993 |
| | BHCO Paired – Chip Paired | 0.09 | 0.994 |
| | BHCO Paired – Seet Paired | -1.24 | 0.427 |
| | BHCO Paired – WOTH Paired | 0.02 | 0.999 |
| | Seet Paired – BLJA Paired | 1.37 | 0.355 |
| | Seet Paired – Chip Paired | 1.39 | 0.343 |
| | Seet Paired – WOTH Paired | 1.28 | 0.404 |

**Supplementary Table 5.** Pair-wise comparisons of chip data by pairing status and treatments: Brown-headed cowbird chatters (BHCO), yellow warbler seet calls (Seet), yellow warbler chip calls (Chip), blue jay calls (BLJA), and wood thrush song (WOTH). Significant *post hoc* differences are represented with asterisks (*** $P < 0.001$, ** $P < 0.01$, * $P < 0.05$)

| Comparisons | | z ratio | *P* value |
|---|---|---|---|
| Treatment *post hoc* - Paired Males | BHCO Paired – BLJA Paired | -3.49 | 0.004** |
| | BHCO Paired – Chip Paired | -2.91 | 0.029* |
| | BHCO Paired – Seet Paired | -0.81 | 0.925 |
| | BHCO Paired – WOTH Paired | 2.76 | 0.045* |
| | BLJA Paired – Chip Paired | 0.60 | 0.975 |
| | BLJA Paired – Seet Paired | 2.55 | 0.078 |
| | BLJA Paired – WOTH Paired | 4.55 | <0.001*** |
| | Chip Paired – Seet Paired | 2.00 | 0.264 |
| | Chip Paired – WOTH Paired | 4.29 | <0.001*** |
| | Seet Paired – WOTH Paired | 3.21 | 0.011* |
| Treatment *post hoc* - Unpaired Males | BHCO Paired – BLJA Paired | 1.01 | 0.848 |
| | BHCO Paired – Chip Paired | -1.87 | 0.332 |
| | BHCO Paired – Seet Paired | 0.60 | 0.974 |
| | BHCO Paired – WOTH Paired | 1.50 | 0.562 |
| | BLJA Paired – Chip Paired | -2.88 | 0.032* |
| | BLJA Paired – Seet Paired | -0.48 | 0.989 |
| | BLJA Paired – WOTH Paired | 0.61 | 0.973 |
| | Chip Paired – Seet Paired | 2.74 | 0.048* |
| | Chip Paired – WOTH Paired | 3.02 | 0.021* |
| | Seet Paired – WOTH Paired | 1.06 | 0.826 |
| Pairing Status *post hoc* | BHCO,Paired - BHCO,Unpaired | 0.69 | 0.486 |
| | Seet,Paired - Seet,Unpaired | 2.13 | 0.033* |
| | BLJA,Paired - BLJA,Unpaired | 4.21 | < 0.001*** |
| | Chip,Paired - Chip,Unpaired | 1.48 | 0.136 |
| | WOTH,Paired - WOTH,Unpaired | -0.61 | 0.538 |

**Supplementary Table 6.** Percentage of trials per playback treatment that non-focal species responded aggressively to playback treatments: Brown-headed cowbird chatters (BHCO), blue jay calls (BLJA), yellow warbler seets (SEET), red- winged blackbird chatters (RWBL), and wood thrush song (WOTH).

| | Playbacks | | | | |
|---|---|---|---|---|---|
| Non-focal species | Brown-headed cowbird chatter | Blue jay calls | Yellow warbler chips | Yellow warbler seets | Wood thrush songs |
| Male brown-headed cowbird | 59.46% | 2.94% | 3.23% | 5.88% | 0.0% |
| Female brown-headed cowbird | 54.05% | 0.0% | 3.23% | 0.0% | 0.0% |
| Gray catbird | 16.22% | 20.59% | 9.68% | 2.94% | 2.86% |
| Indigo bunting | 8.11% | 11.76% | 29.03% | 5.88% | 0.0% |
| Northern cardinal | 8.11% | 0.0% | 0.0% | 0.0% | 0.0% |
| Willow flycatcher | 8.11% | 2.94% | 3.23% | 0.0% | 0.0% |
| Song sparrow | 8.11% | 2.94% | 3.23% | 2.94% | 0.0% |
| Common grackle | 5.41% | 8.82% | 0.0% | 0.0% | 0.0% |
| American robin | 2.70% | 14.71% | 3.23% | 0.0% | 0.0% |
| Orchard oriole | 2.70% | 2.94% | 0.0% | 0.0% | 0.0% |
| Baltimore oriole | 2.70% | 5.88% | 0.0% | 0.0% | 0.0% |
| Cedar waxwing | 2.70% | 5.88% | 0.0% | 0.0% | 0.0% |
| Blue-gray gnatcatcher | 2.70% | 2.94% | 0.0% | 0.0% | 0.0% |
| Warbling vireo | 2.70% | 2.94% | 6.45% | 0.0% | 0.0% |
| Chipping sparrow | 2.70% | 0.0% | 0.0% | 0.0% | 0.0% |
| Blue jay | 0.0% | 29.41% | 0.0% | 0.0% | 0.0% |
| Common yellowthroat | 0.0% | 5.88% | 12.90% | 2.94% | 0.0% |
| Field sparrow | 0.0% | 2.94% | 3.23% | 0.0% | 0.0% |
| Eastern kingbird | 0.0% | 2.94% | 0.0% | 0.0% | 0.0% |
| American goldfinch | 0.0% | 0.0% | 6.45% | 2.94% | 0.0% |
| Tufted titmouse | 0.0% | 0.0% | 3.23% | 0.0% | 0.0% |
| Least flycatcher | 0.0% | 2.94% | 0.0% | 0.0% | 0.0% |
| Carolina wren | 0.0% | 2.94% | 0.0% | 0.0% | 0.0% |
| Wood thrush | 0.0% | 0.0% | 0.0% | 0.0% | 20.0% |

**Supplementary Table 7.** Pair-wise comparisons of non-zero latency data by treatments: Brown-headed cowbird chatters (BHCO), blue jay calls (BLJA), yellow warbler seets (SEET), red-winged blackbird chatters (RWBL), and wood thrush song (WOTH).

| | | z value | P value |
|---|---|---|---|
| Males | BLJA x BHCO | -0.64 | 0.967 |
| | RWBL x BHCO | -1.26 | 0.706 |
| | SEET x BHCO | -0.87 | 0.904 |
| | WOTH x BHCO | 5.12 | < 0.001*** |
| | RWBL x BLJA | -0.46 | 0.99 |
| | SEET x BLJA | -0.10 | 1.00 |
| | WOTH x BLJA | 5.18 | < 0.001*** |
| | SEET x RWBL | 0.38 | 0.995 |
| | WOTH x RWBL | 6.25 | < 0.001*** |
| | WOTH x SEET | 5.59 | < 0.001*** |
| Females | BLJA x BHCO | 0.63 | 0.970 |
| | RWBL x BHCO | -1.91 | 0.305 |
| | SEET x BHCO | 0.14 | 1.00 |
| | WOTH x BHCO | 5.72 | <0.001*** |
| | RWBL x BLJA | -2.5 | 0.090 |
| | SEET x BLJA | -0.47 | 0.990 |
| | WOTH x BLJA | 4.54 | <0.001*** |
| | SEET x RWBL | 2.07 | 0.232 |
| | WOTH x RWBL | 7.45 | <0.001*** |
| | WOTH x SEET | 5.32 | <0.001*** |

**Supplementary Table 8.** Pair-wise comparisons of closest approach data by treatments: Brown-headed cowbird chatters (BHCO), blue jay calls (BLJA), yellow warbler seets (SEET), red-winged blackbird chatters (RWBL), and wood thrush song (WOTH).

| | | z value | P value |
|---|---|---|---|
| Males | BLJA x BHCO | 1.68 | 0.442 |
| | RWBL x BHCO | -1.35 | 0.658 |
| | SEET x BHCO | 2.90 | 0.029* |
| | WOTH x BHCO | 4.43 | <0.001*** |
| | RWBL x BLJA | -2.84 | 0.035* |
| | SEET x BLJA | 1.01 | 0.846 |
| | WOTH x BLJA | 2.27 | 0.152 |
| | SEET x RWBL | 4.18 | <0.001*** |
| | WOTH x RWBL | 5.81 | <0.001*** |
| | WOTH x SEET | 1.25 | 0.718 |
| Females | BLJA x BHCO | 1.05 | 0.829 |
| | RWBL x BHCO | -1.61 | 0.487 |
| | SEET x BHCO | 1.21 | 0.744 |
| | WOTH x BHCO | 2.10 | 0.219 |
| | RWBL x BLJA | -2.56 | 0.076 |
| | SEET x BLJA | 0.14 | 0.999 |
| | WOTH x BLJA | 0.91 | 0.890 |
| | SEET x RWBL | 2.67 | 0.057 |
| | WOTH x RWBL | 3.57 | 0.003** |
| | WOTH x SEET | 0.71 | 0.954 |

**Supplementary Table 9.** Pair-wise comparisons of calling rate by treatments: Brown-headed cowbird chatters (BHCO), blue jay calls (BLJA), yellow warbler seets (SEET), red- winged blackbird chatters (RWBL), and wood thrush song (WOTH).

| | | z value | P value |
|---|---|---|---|
| 2018 Males | RWBL x BHCO | 4.37 | <0.001*** |
| | SEET x BHCO | -0.27 | 0.992 |
| | WOTH x BHCO | -3.04 | 0.011* |
| | SEET x RWBL | -4.45 | <0.001*** |
| | WOTH x RWBL | -8.16 | <0.001*** |
| | WOTH x SEET | -2.66 | 0.037* |
| 2019 Males | BLJA x BHCO | -1.74 | 0.405 |
| | RWBL x BHCO | 2.73 | 0.048* |
| | SEET x BHCO | -0.44 | 0.991 |
| | WOTH x BHCO | -7.63 | <0.001*** |
| | RWBL x BLJA | 4.35 | <0.001*** |
| | SEET x BLJA | 1.13 | 0.786 |
| | WOTH x BLJA | -5.67 | <0.001*** |
| | SEET x RWBL | -2.98 | 0.023* |
| | WOTH x RWBL | -9.76 | <0.001*** |
| | WOTH x SEET | -6.29 | <0.001*** |
| 2019 Females | BLJA x BHCO | 1.78 | 0.381 |
| | RWBL x BHCO | 4.78 | < 0.001*** |
| | SEET x BHCO | 1.64 | 0.466 |
| | WOTH x BHCO | -1.82 | 0.360 |
| | RWBL x BLJA | 2.79 | 0.041* |
| | SEET x BLJA | -0.12 | 0.999 |
| | WOTH x BLJA | -3.53 | 0.003** |
| | SEET x RWBL | -2.86 | 0.033* |
| | WOTH x RWBL | -6.43 | < 0.001*** |
| | WOTH x SEET | -3.20 | 0.011* |

**Supplementary Table 10.** Pair-wise comparisons of latency data by treatments: Brown-headed cowbird chatters (BHCO), yellow warbler seet calls (Seet), blue jay calls (BLJA), and wood thrush song (WOTH). Significant *post hoc* differences are represented with asterisks (*** *P* < 0.001, ** *P* < 0.01, * *P* < 0.05)

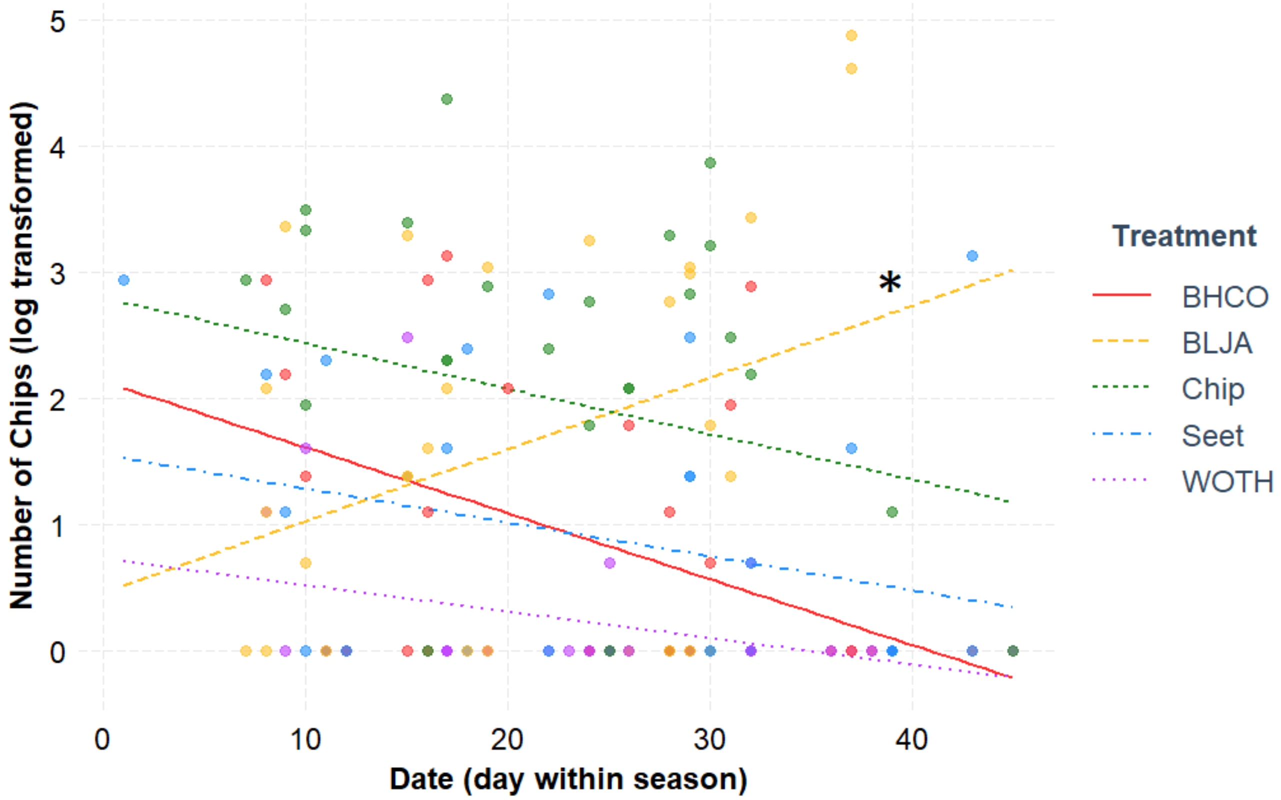
| Comparisons | | z ratio | *P* value |
|---|---|---|---|
| Treatment *post hoc* - Males | BHCO Paired – BLJA Paired | 4.44 | <0.001*** |
| | BHCO Paired – Seet Paired | -1.80 | 0.273 |
| | BHCO Paired – WOTH Paired | -2.02 | 0.180 |
| | BLJA Paired – Seet Paired | -6.30 | <0.001*** |
| | BLJA Paired – WOTH Paired | -6.25 | <0.001*** |
| | Seet Paired – WOTH Paired | -3.99 | 0.979 |
| Treatment *post hoc* - Females | BHCO Paired – BLJA Paired | 3.14 | <0.01** |
| | BHCO Paired – Seet Paired | 1.13 | 0.999 |
| | BHCO Paired – WOTH Paired | 0.005 | 0.999 |
| | BLJA Paired – Seet Paired | -3.06 | 0.011* |
| | BLJA Paired – WOTH Paired | -2.92 | 0.018* |
| | Seet Paired – WOTH Paired | -0.11 | 0.999 |

**Supplementary Table 11.** Pair-wise comparisons of closest approach data by treatments: Brown-headed cowbird chatters (BHCO), yellow warbler seet calls (Seet), blue jay calls (BLJA), and wood thrush song (WOTH). Significant *post hoc* differences are represented with asterisks (*** *P* < 0.001, ** *P* < 0.01, * *P* < 0.05)

| Comparisons | | z ratio | *P* value |
|---|---|---|---|
| Treatment *post hoc* - Males | BHCO Paired – BLJA Paired | -1.33 | 0.544 |
| | BHCO Paired – Seet Paired | -2.73 | 0.037* |
| | BHCO Paired – WOTH Paired | -3.81 | 0.001** |
| | BLJA Paired – Seet Paired | -1.34 | 0.536 |
| | BLJA Paired – WOTH Paired | -2.59 | 0.053* |
| | Seet Paired – WOTH Paired | -1.35 | 0.528 |

**Supplementary Table 12.** Pair-wise comparisons of alarm calling data by treatments: Brown-headed cowbird chatters (BHCO), yellow warbler seet calls (Seet), blue jay calls (BLJA), and wood thrush song (WOTH). Significant *post hoc* differences are represented with asterisks (*** $P < 0.001$, ** $P < 0.01$, * $P < 0.05$)

| Comparisons | | z ratio | *P* value |
|---|---|---|---|
| Treatment *post hoc* – All males | BHCO Paired – BLJA Paired | -2.53 | 0.055* |
| | BHCO Paired – Seet Paired | 0.73 | 0.881 |
| | BHCO Paired – WOTH Paired | 1.34 | 0.533 |
| | BLJA Paired – Seet Paired | 3.31 | <0.01** |
| | BLJA Paired – WOTH Paired | 3.75 | <0.001*** |
| | Seet Paired – WOTH Paired | 0.69 | 0.900 |
| Treatment *post hoc* – Males without any nests with eggs | BHCO Paired – BLJA Paired | -2.64 | 0.040* |
| | BHCO Paired – Seet Paired | 0.52 | 0.952 |
| | BHCO Paired – WOTH Paired | 0.93 | 0.788 |
| | BLJA Paired – Seet Paired | 3.40 | <0.01** |
| | BLJA Paired – WOTH Paired | 3.71 | <0.001*** |
| | Seet Paired – WOTH Paired | 0.43 | 0.971 |
| Treatment *post hoc* - Females | BHCO Paired – BLJA Paired | -5.84 | <0.001*** |
| | BHCO Paired – Seet Paired | 0.05 | 0.999 |
| | BHCO Paired – WOTH Paired | -2.11 | 0.147 |
| | BLJA Paired – Seet Paired | 5.99 | <0.001*** |
| | BLJA Paired – WOTH Paired | 3.33 | <0.01** |
| | Seet Paired – WOTH Paired | 2.16 | 0.133 |



**Supplementary Figure 1.** Chip production by all yellow warbler males as a function of date (ordinal day in season) and playback treatment. Significant *post hoc* differences in slope for each treatment across the breeding season are represented with asterisks (* $P < 0.05$).