Risk assessment during nest defense against three simulated predators by female northern house wrens (*Troglodytes aedon aedon*)

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Abstract

Offspring predation is one of the greatest obstacles to an organism's reproductive success, but parents vary in the strength of their response to potential predators. One explanation for this variable investment is that defending current offspring has the potential to lower future reproductive success if the predator is also capable of injuring or killing the parent. House wrens (*Troglodytes aedon aedon*) are cavity-nesting songbirds that defend against multiple species of nest predators including small mammals, birds of prey, and snakes. Here, we used three different predator decoys: two nest predators - an eastern chipmunk (*Tamias striatus*) and an eastern ratsnake (*Pantherophis alleghaniensis*) - as well as a predator of both offspring and adults - a juvenile Cooper's hawk (*Accipiter cooperi*) - to elicit nest defense and test whether females use risk assessment to modulate their antipredator behavior. We found that antipredator behaviors were not significantly different between the two nest predators, which posed a high risk to the nestlings, but lower risk to the parents as neither species frequently captures adult wrens. Females also did not differ significantly in their responses to the snake between years, suggesting that the population-level response might be consistent through time. However, female wrens never dove at or attacked the Cooper's hawk despite frequently attacking both the snake and chipmunk decoys. Collectively, these results show that female house wrens exhibit plasticity in their antipredator behavior, and they respond to different types of predators in a way that could maximize lifetime fitness while risking the loss of their current offspring.

Introduction

Parental investment can be defined as any behaviors that increase an offspring's chance of survival while simultaneously decreasing the parent's ability to invest in other offspring both currently and in the future (Trivers 1974; Montgomerie and Weatherhead 1988; Clutton-Brock 1991). This tradeoff between current and future reproduction creates a parent-offspring conflict (*sensu* Trivers 1974) because the optimal parenting strategy, one that maximizes the parent's lifetime fitness, often requires submaximal investment in the current offspring, which can decrease their survival and fitness. The amount of parental investment varies widely both within and among species (Fontaine and Martin 2006; Dulac et al. 2014), and this variation can be further explained by differences in life history strategies (Ricklefs 1977; Montgomerie and Weatherhead 1988; Clutton-Brock 1991; Ghalambor and Martin 2001). For example, both adult mortality rates and offspring number and age should correlate positively with parental investment because species with shorter lifespans and larger current investments will have fewer reproductive opportunities in the future (Caro 2005).

Optimally modulating parental investment is particularly critical in the context of antipredator behavior, as parents that vigorously defend their young risk completely eliminating their future reproduction if they are mortally injured or killed. On the other hand, parents that limit their antipredator behavior are more likely to lose their current offspring as predation is one of the most common causes of reproductive failure across species (Ricklefs 1977; Caro 2005; Martin and Briskie 2009; Ibáñez-Álamo et al. 2015). The most successful parents should be those that exhibit plastic antipredator behavior that maximizes their lifetime reproductive success, and selection should favor individuals with accurate risk assessment during encounters with predators near their offspring (Montgomerie and Weatherhead 1988).

An abundance of evidence supports the hypothesis that animals from across the taxonomic spectrum use risk assessment to adjust their antipredator behavior relative to the perceived threat level (reviewed in Caro 2005; Lima 2009). Species with altricial young, such as passerine songbirds, are particularly vulnerable to predation at the nest because their offspring are incapable of self-defense or escape without parental assistance. Consequently, parents respond to different predation pressures with both proactive and reactive behavior. When the perceived risk of predation is high, parents can proactively reduce egg or clutch sizes (Fontaine and Martin 2006; Morosinotto et al. 2010; Travers et al. 2010) or limit visits to the nest, including offspring provisioning (Fontaine and Martin 2006; Ghalambor et al. 2013; Dudeck et al. 2018), which minimizes their potential energetic losses and draws less attention to the nest site. Alternatively, parents may simply avoid nesting in locations with high predator abundance or even forgo reproduction entirely (Spaans et al. 1998; Morosinotto et al. 2010; Szymkowiak and Thomson 2019).

When a predator appears at the nest site, parents can react in ways that might divert the predator's attention toward the parents rather than the offspring such as alarm calling or visual distraction displays (e.g. broken-wing display; de Framond et al. 2022). Although these behaviors increase risk to the parents, they are often less costly than more aggressive defense behaviors such as diving at or hitting the predator in an attempt to physically drive it away from the offspring. Studies from multiple species have shown that parents exhibit plasticity in their antipredator responses and adjust their behavior according to factors such as the distance between the predator and the offspring (Colombelli-Négrel et al. 2010; Fernández and Carro 2022) and the relative risks posed to both adults and offspring by different types of predators (Gottfried 1979; Winkler 1992; Mahr et al. 2015; Duré Ruiz et al. 2018; Teunissen et al. 2020).

We studied risk assessment by female northern house wrens (*Troglodytes aedon aedon*) during simulated interactions with predators at their nest. House Wrens are socially monogamous, cavity-nesting songbirds that readily use artificial nest boxes to raise clutches of up to 8 offspring (Johnson 2020). The guild of house wren nest predators is diverse and includes mammals ranging in size from mice (e.g., *Peromyscus* spp.) to bears (*Ursus spp.*), songbirds (e.g., *Cyanocitta* spp.), woodpeckers (e.g., *Melanerpes* spp.), raptors, and snakes (Johnson 2020). Previous research in both the northern and southern house wren subspecies has found evidence for risk assessment and plasticity in parental behavior in response to predation pressure. For example, wren parents produced more alarm calls when a snake model was placed closer to their nest (Fernández and Carro 2022), consistent with an ability to perceive levels of risk. After interacting with a simulated nest predator, wren parents have also been shown to reduce their offspring provisioning (Freed 1981; Ghalambor et al. 2013) and take longer to re-enter the nest box when compared to control observations (Corral et al. 2013; Fernandez and Llambías 2013; Fernández et al. 2015; Duré Ruiz et al. 2018). When presented with different simulated species of raptors of varying threat levels, male and female southern house wrens (*Troglodytes aedon musculus*) took longer to resume normal parental behavior after encountering a predator of both adults and nestlings than a predator of nestlings only (Duré Ruiz et al. 2018). This result is consistent with house wrens assessing the threat posed by a heterospecific bird at their nest and responding in a potentially adaptive way by behaving more cautiously in the presence of a predator of adult wrens.

Here, we expand on these observations by testing whether wren parents assess risks posed by both avian and non-avian predators and scale their aggressive nest defense behavior accordingly. Over two breeding seasons, we placed three simulated predators of varying risks on

top of house wren nest boxes during the early nestling stage. One predator, a rubber eastern ratsnake decoy (*Pantherophis alleghaniensis*), was presented in both years, while the second predator differed between years and consisted of a rubber eastern chipmunk decoy (*Tamias striatus*) in year one and a taxidermied juvenile Cooper's Hawk (*Accipiter cooperii*) in year two. We assumed that the ratsnake and chipmunk decoys posed a high risk to nestlings, but a low-to-moderate risk to adult wrens, while the hawk posed a high risk to adults, but a low-to-moderate risk to young nestlings of limited mobility that were protected inside the nest box (Johnson 2020). If the hypothesis that house wrens use risk assessment is supported, we predicted that female wrens would respond equally aggressively to the ratsnake and chipmunk decoys, which are similar in threat level to both nestlings and adults. Female responses to the hawk were predicted to be less aggressive overall due to the higher threat that the hawk posed to adults. We also predicted a positive relationship between nest defense behavior and clutch size across predator types because larger reproductive investments are more costly to lose.

Methods

Study Sites

We monitored the breeding success of northern house wrens (*Troglodytes aedon aedon*) at 137 nest boxes divided between six sites in Delaware and Union Counties, Ohio, USA during the 2020 and 2021 breeding seasons. The nest boxes varied slightly in dimensions due to landowner preferences, but the average internal dimensions were 110 x 115 x 195 mm and the entrance hole size was 38 mm. Each nest box was mounted on a conduit pipe approximately 1.75 m above the ground, and the pipe was covered in axle grease to limit access by predators. The

majority of the boxes were located in edge habitat near the transition from grass or field to forest, but a small subset were located in suburban backyards (*N*=7).

Banding of Adults and Nestlings

We captured male house wrens using mist nets and a brief playback of conspecific song. Each male was banded with a USFWS aluminum leg band and a unique combination of 2-3 additional colored bands for identification from a distance. We also collected morphological measurements and a small blood sample for an unrelated study. Any females that were captured were rapidly banded and immediately released to limit the effects of handling on subsequent behavior. All subjects were captured and banded at least 24 hours before any behavioral trials.

We also monitored the number of eggs laid, hatching success, and offspring development for every nest box. We counted the number of nestlings at the end of each behavioral trial (see below), and banded nestlings when they were 8 days old. Each nestling received a single USFWS aluminum band, and we collected morphological measurements and a small blood sample. After banding, we passively monitored each nest box until all visible and acoustic activity ceased. Unless the contents of the nest suggest otherwise (i.e. presence of dead nestlings and/or their parts), we assumed that empty nests successfully fledged.

Experiment 1: Chipmunk v. Snake Decoy

In 2020, we conducted behavioral trials at 47 nest boxes containing nestlings between the ages of 2-7 days old. Two life-like rubber predator decoys, an eastern chipmunk (*Tamias striatus*; Safari Ltd.) and an eastern ratsnake (*Pantherophis alleghaniensis*; Flybanboo), were used to elicit a nest defense behavior. We waited for each female to enter or land on the nest box before flushing

them away during setup. One predator was placed on top of the nest box, and then on the following day the other predator was presented in a counterbalanced order to control for order effects.

All trials occurred between 7:00-13:00, but we were unable to conduct trials exactly 24 hours apart due to the distant locations of our field sites and unpredictability in how rapidly females would return to their boxes. Trials were recorded with a video camera mounted on a tripod placed roughly 2-3 meters away from the nest box to later confirm behaviors and differentiate between the male and female wren in cases of uncertainty. Each decoy was attached to a fishing line, and at the completion of the trial we used a fishing rod to rapidly reel the decoy away from the box. This approach eliminated the need for the observer to approach the box and remove the decoy. We allowed the video camera to continue to record for an additional 20 minutes to measure the amount of time it took for the female to re-enter the nest box after removal of the decoy (N=36).

We quantified the nest defense behavior of female house wrens for a total of seven minutes after the decoy was placed on the nest box. We measured the number of hits and flyovers, which were defined as any contact with the decoy and flights over the decoy, respectively. We also measured the amount of time females spent within five meters of the nest box, the amount of time spent physically on or in the box, and their closest approach to the decoy. Any individuals that made contact with the decoy received a closest approach score of zero. We also noted whether or not the female produced alarm calls, and whether the male was present during trial. Neighboring conspecifics and heterospecifics sometimes responded to the predator decoys by approaching, alarm calling, and in rare cases flying over and hitting the

decoy. During each trial, we noted the number of individuals of each species that approached within 5 meters of the decoy and either oriented towards the decoy or produced alarm calls.

Behavioral data from both predator trials were excluded from our final analysis if either of the decoys were knocked off the box during either trial (*N*=3). If the decoy was knocked over but remained on the box, the trials were not excluded (*N*=5). We also excluded trials if the female did not return to the box within seven minutes of initiating the trial (*N*=6). In response to this limitation, we adjusted our protocol early in the season to remove the decoy and reset the trial if the female did not return within 4 minutes after the trial was initiated.

Experiment 2: Hawk v. Snake Decoy

In 2021, we replicated this experiment in the same house wren population, but we replaced the chipmunk decoy with a taxidermied, juvenile Cooper's hawk (*Accipiter cooperii*). The hawk was mounted with wire onto a wooden plank, and two large binder clips were attached to the bottom of the plank with jewelry wire. This design allowed the decoy to be quickly attached and removed from the roof of the nest box while eliminating the possibility that it could be knocked off the box.

We conducted behavioral trials at 41 nest boxes with nestlings aged 2-7 days. A different observer (JHF) scored the trials in 2021, but she was trained by the observer (RCE) from 2020 to ensure consistency between years. All of our sampling methods were replicated from experiment 1, but we were unable to continue to use the fishing rod and line to rapidly remove the decoys. As a result, one of the observers quickly ran to the box and removed the decoy at the end of each seven-minute trial. The video camera continued to record for an additional 20 minutes (*N*=38), but the approach of the observer may have affected the return to normal behavior.

It is difficult to determine how many females were sampled in both experiments because only 13 of 47 females (27.7%) were banded in experiment one. Of those banded females, only one (7.7.% return rate) was definitely sampled in experiment two. It is likely that some of the unbanded females also returned to be sampled again, and some inferences can be drawn from males, which were banded at much higher rates. In experiment one, 43 of 47 males (91.5%) were banded and eight males returned to be sampled again in experiment two (18.5% return rate). Based on these data, it seems likely that 10-20% of females were sampled in both experiments.

All procedures were reviewed and approved by the Ohio Wesleyan University Institutional Animal Care and Use Committee (Protocol Numbers: Spring_2020-21-01_Reichard and 05-2021-06).

Statistical analysis

For each year of data, we generated linear mixed models in R version 4.1.3 (R Core Team 2022); packages: "lme4," "car") to test whether female behaviors including flyovers, hits, closest approach to the decoy, time spent within five meters of the decoy, and latency to re-enter the nestbox after removal of the predator differed between predator types. Measurements of time spent on or inside the box were excluded due to extremely low rates of occurrence in both years. Each model included female behavior as the dependent variable with predator type, treatment order, and their interaction as fixed effects. Day of the year and female ID were included as random effects to control for seasonal shifts in behavior and repeated sampling of the same individuals.

We used a similar linear mixed model approach to determine whether the number of neighboring conspecific and heterospecific individuals and the number of heterospecific species

that responded to the trials differed between predator types in both years. The total number of conspecifics, total number of heterospecifics, and the number of heterospecific species were used as dependent variables in three separate models. We included predator type, treatment order and their interaction as fixed effects and day of the year and female ID as random effects.

We also used linear mixed models to conduct inter-annual comparisons of female behavior for the common predator present during both years, the eastern ratsnake. Due to the change in retrieval protocol of the predator models between the two years (see above), we were unable to compare latency to re-enter the box inter-annually. Data points for this analysis were excluded if the subject was also excluded from the intra-annual aggression analysis (see above). In each model, female behavior was the dependent variable and year was the fixed effect. Treatment order and day of the year were also included as random effects.

We used linear regressions (Base R package) to test for relationships between the antipredator behaviors and clutch size. In addition, we used linear mixed models to compare antipredator behaviors between nests that fledged and nests that failed. Nest outcome was included as a fixed effect with day of the year and female ID as random effects.

Results

Experiment 1: Chipmunk v. Snake Decoy

Female wrens exhibited no significant differences in the number of hits (χ^2_1=0.01, P=0.91), number of flyovers (χ^2_1=0.0001, P>0.99), time spent within 5 meters of the nest box (χ^2_1=0.46, P=0.50), or in their closest approach (χ^2_1=1.45, P=0.23) to the snake and chipmunk decoys (Figure 1A-D). After the predator decoys were removed, we found no significant difference between predator types in the amount of time females took to re-enter the nest box

(χ^2_1=0.06, *P*=0.81; Figure 2A). For all five behaviors, we found no evidence for significant effects of treatment order (P>0.14 in all cases) or significant interactions between treatment order and predator type (P>0.09 in all cases).

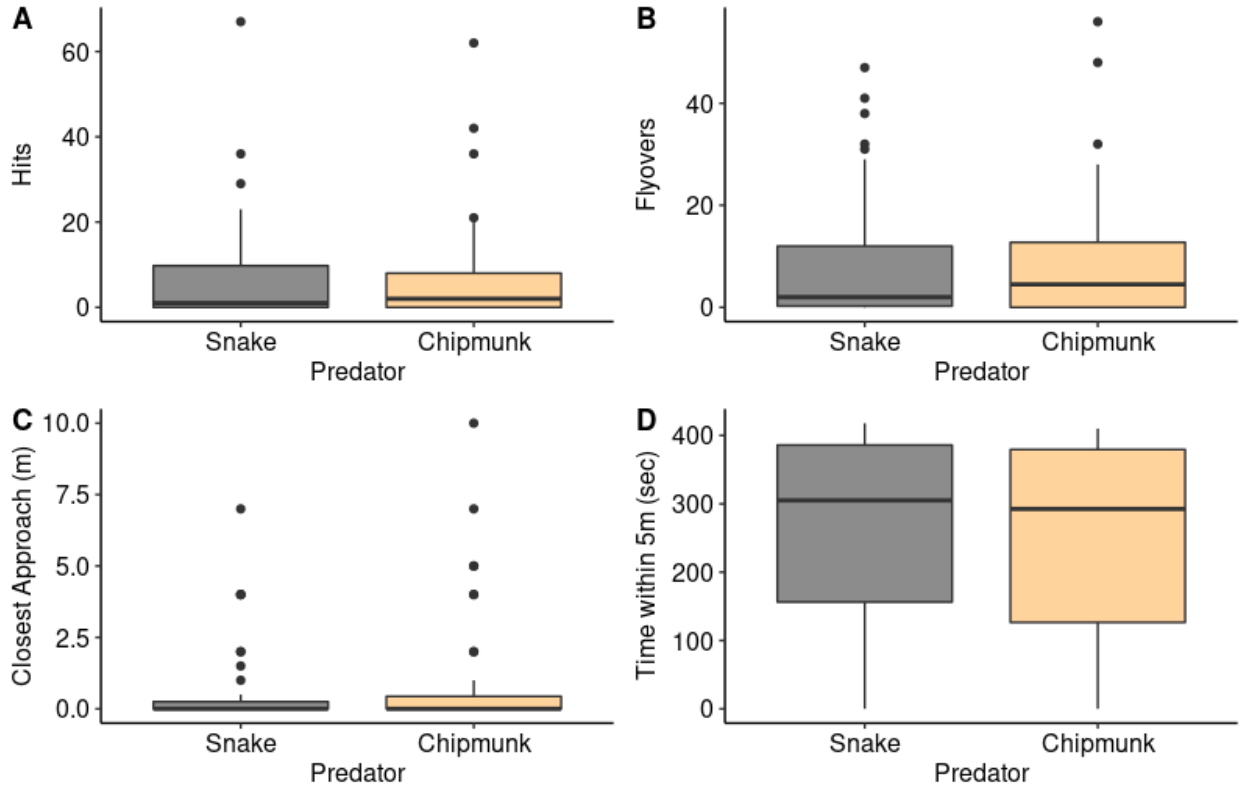

Figure 1 — Responses of female house wrens to a snake or chipmunk decoy on top of their nest box during a seven minute trial. There were no significant differences in the number of (A) hits (P=0.91), (B) flyovers (P>0.99), (C) how closely females approached each model (P=0.23), or (D) how much time females spent within 5 m of each model (P=0.50). The thick center line represents the median, and the box encloses the interquartile range. Whiskers show the range of data within 1.5 times the interquartile range, and dots are data points exceeding that range.

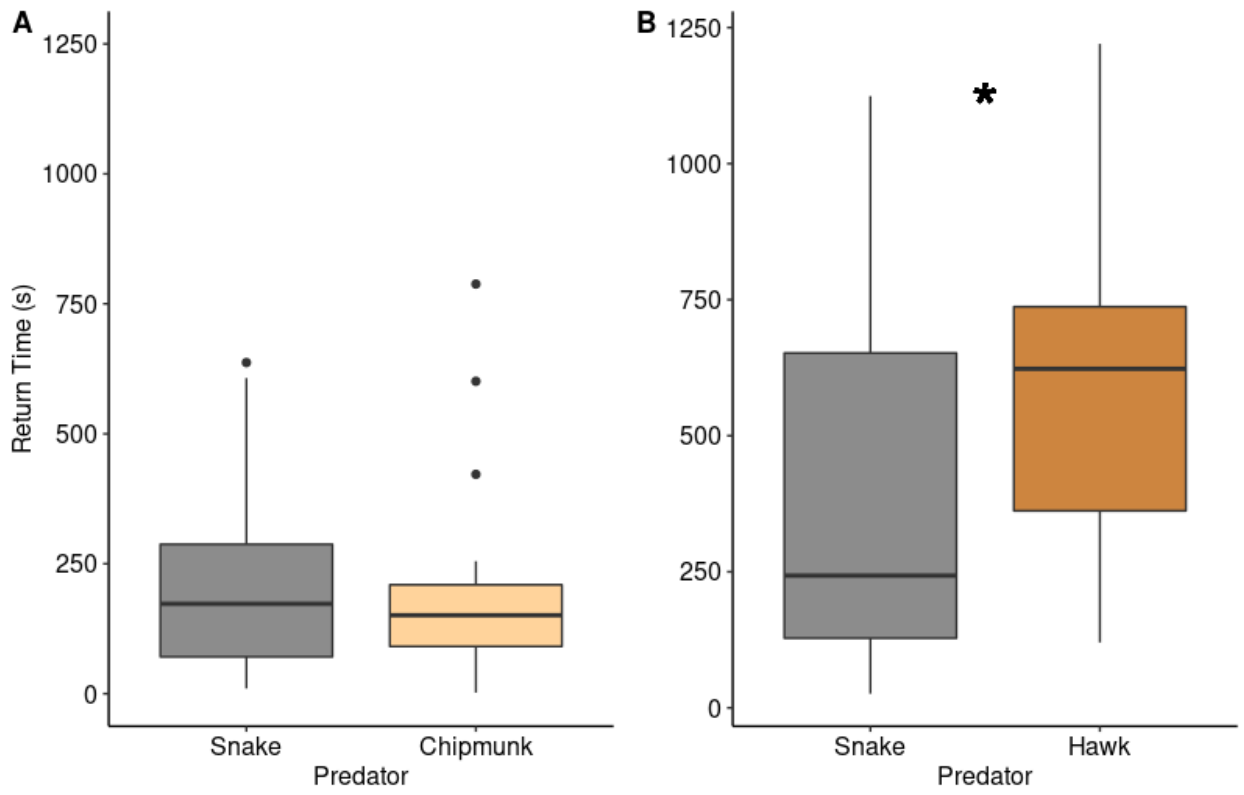

*Figure 2 — Female house wrens modulate how quickly they resume normal behavior after encountering different nest predator decoys. (A) There was no detectable difference in how long it took female wrens to resume normal behavior after encountering a snake decoy versus a chipmunk decoy (P=0.81). (B) Females took significantly longer to resume normal behavior after encountering a hawk decoy than after encountering a snake decoy (P=0.03). Box plots as in Fig. 1. *P<0.05.*

The number of conspecific (χ^2_1=0.28, *P*=0.59) and heterospecific individuals (χ^2_1=0.49, *P*=0.48) and the number of heterospecific species (χ^2_1=0.06, *P*=0.81) that responded to the predator decoys did not differ detectably between the two predator types. We also found no significant effects of treatment order on any of these measurements (P>0.08 in all cases). However, we did note there was a significant predator type by treatment order interaction on the

number of heterospecific individuals that responded to the predator decoys (χ^2_1=4.23, P=0.04). More heterospecifics responded to the snake when it was presented during the first trial, but there was a stronger heterospecific response to the chipmunk during the second trial.

We found no relationship between clutch size and the number of hits, number of flyovers, time spent within 5 meters of the nest box, closest approach, or latency for female wrens to return to the nest box after the trial (P>0.10 in all cases; Table S1, Supplemental Materials). We also found no significant differences in nest defense behavior between nests that fledged (N=21) and nests that failed (N=4; P>0.42 for all behaviors). The sample size for this comparison is reduced (N=25) because multiple nests remained active after the end of our data collection and their end point is unknown.

Experiment 2: Hawk v. Snake Decoy

Similar to Experiment 1, female wrens frequently performed hits and flyovers while defending against the snake decoy, with 25 out of 41 females hitting the snake model at least once and 38 out of 41 females flying over at least once. Notably, none of the female wrens performed hits or flyovers while defending against the hawk model (Figure 3). Statistically, females performed significantly more hits (χ^2_1=14.72, P < 0.001) and flyovers (χ^2_1=41.20, P < 0.001) in response to the snake decoy (Figure 3A-B). Females approached significantly closer to the snake (χ^2_1=116.9, P < 0.001; Figure 3C), but the amount of time spent within 5 meters was not significantly different (χ^2_1=3.04, P=0.08) despite a marginal trend towards more time in close proximity to the snake (Figure 3D). We found no evidence for an effect of treatment order (P>0.15 in all cases) or a predator type by treatment order interaction (P>0.16 in all cases) for total hits, total flyovers, or closest approach, but there was a slight trend towards an order effect

for time spent within 5 meters of the predator decoy (χ^2_1=3.48, *P*=0.06). Female wrens tended to spend more time within 5 meters of the decoy during the second trial than during the first trial regardless of the order in which predators were presented. Finally, after the removal of the predator decoys, female wrens took significantly more time to re-enter the nest box after the hawk trials than after the snake trials (χ^2_1=4.80, *P*=0.03; Figure 2B). There was no detectable effect of treatment order (χ^2_1=0.02, *P*=0.89) or an interaction between order and predator type (χ^2_1=0.07, *P*=0.79) for this behavior.

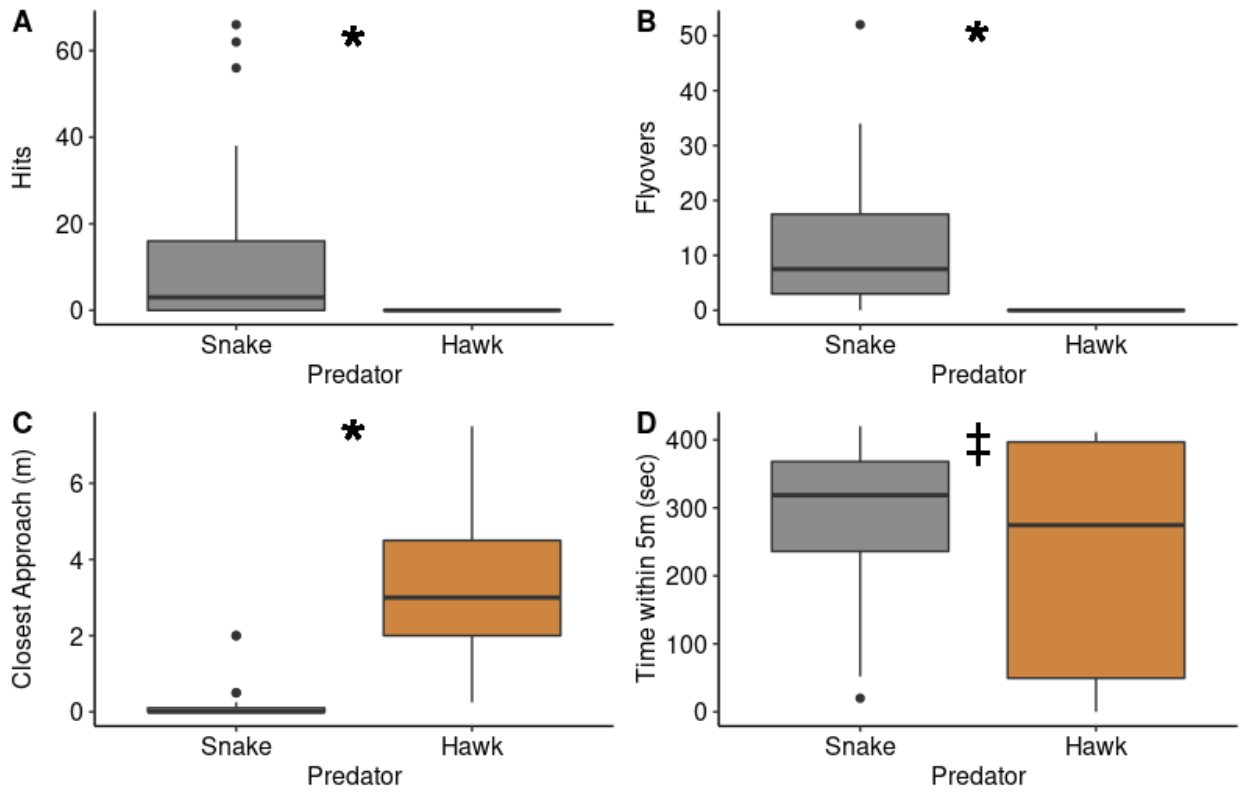

Figure 3 — Responses of female house wrens to a snake or hawk decoy on top of their nest box during a seven minute trial. Female house wrens performed significantly more (A) hits (P<0.001) and (B) flyovers (P<0.001) against the snake decoy than against the hawk decoy. Female wrens also (C) made significantly closer approaches to (P<0.001) and (D) spent more

time within 5 meters of the snake decoy than the hawk decoy (P=0.08). Box plots as in Fig. 1. ∗ P<0.001. ‡ P<0.10.

Significantly more heterospecific individuals responded to the hawk decoy than to the snake decoy (χ^2_1=8.43, *P*=0.004). Furthermore, the number of different heterospecific species that responded to each predator decoy was almost significant (χ^2_1=3.52, *P*=0.06) with the hawk decoy tending to be mobbed by more heterospecific species than the snake. In contrast, significantly more conspecific individuals mobbed the snake decoy than mobbed the hawk (χ^2_1=8.89, *P*=0.003). In contrast to our results from Experiment 1, we found no evidence for an effect of treatment order (*P*>0.25 in all cases) or a predator type by treatment order interaction (*P*>0.19 in all cases) for heterospecifc and conspecific responses to the two decoys.

As in 2020, we found no evidence of a relationship between clutch size and any metrics of nest defense behavior (*P*>0.14 in all cases; Table S1, Supplemental Materials). We were unable to compare nest defense behavior between nests that successfully fledged and those that failed due to a limited sample size of failed nests (*N*=1).

Interannual Comparison — Snake 2020 Data v. Snake 2021 Data

In addition to comparing how female house wrens responded to two different nest predators during a single breeding season, we compared how the wrens responded to the same predator—the snake—during two different breeding seasons. There was no significant difference between the number of hits (χ^2_1=1.10, *P*=0.30), the number of flyovers (χ^2_1=1.06, *P*=0.30), or the time that female wrens spent within 5 meters of the snake decoy (χ^2_1=0.19, *P*=0.67) between years (Figure 4). We found a significant difference (χ^2_1=4.28, *P*=0.04) between the female wrens'

closest approach to the snake decoy in 2020 ($\bar{x}$=0.72 meters) versus in 2021 ($\bar{x}$=0.18 meters; Figure 4). However, after removing two low-responding outliers from the 2020 data set (closest approach = 7 m, 4 m respectively), the difference was no longer significant (χ^2_1=2.36, *P*=0.13; Figure 4).

Because the majority of the female behaviors were not statistically different between the two years, we combined both years of snake response data to increase our sample size and test for a relationship between clutch size and nest defense behavior. However, the results remained unchanged as we found no evidence for any significant relationships between female behavior and clutch size (P>0.32 for all behaviors).

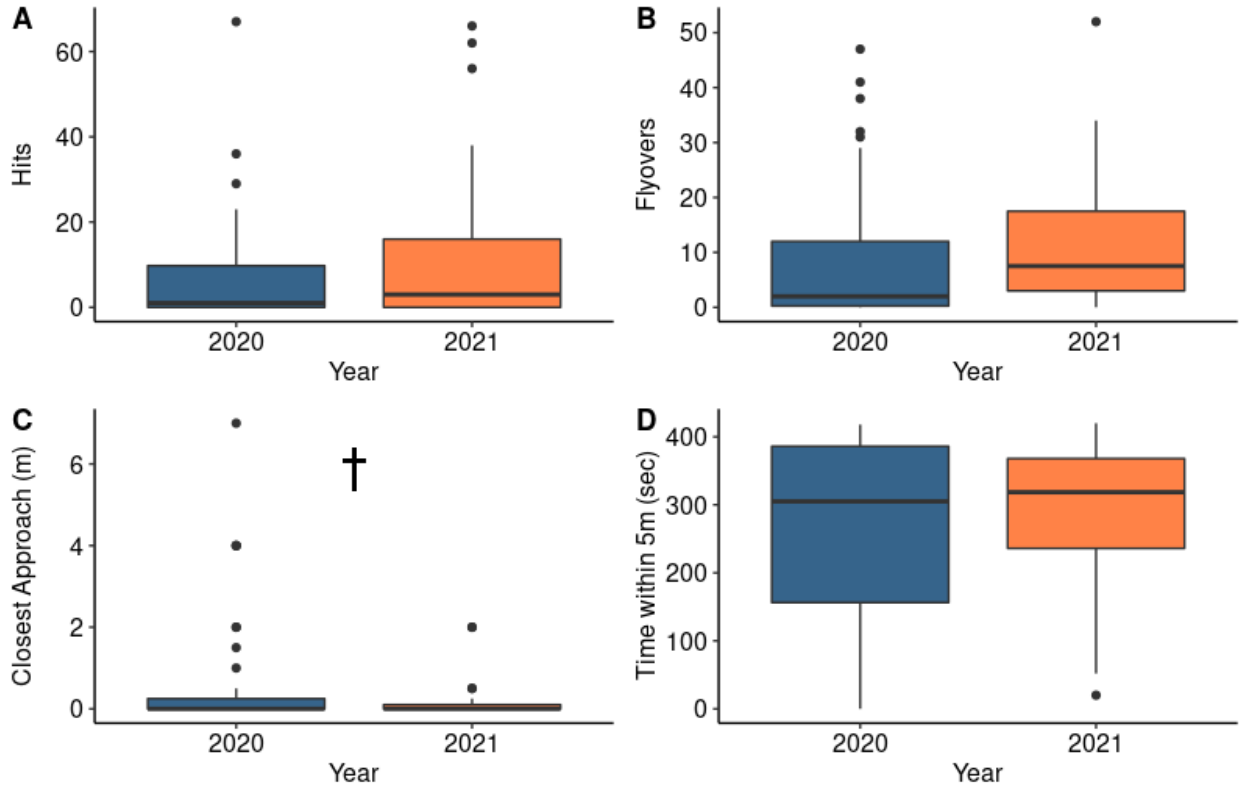

Figure 4 — Interannual comparison of female house wren nest defense behaviors in response to a snake decoy across two breeding seasons. There were no significant differences in (A) the number of hits (P=0.30) or (B) the number of flyovers (P=0.30). (C) Female wrens made significantly closer approaches in 2021 than in 2020 (P=0.04), but when two extreme outliers were removed from the 2020 data set, the difference was no longer significant (P=0.13). (D) There was no significant difference between the amount of time spent within 5 meters of the snake model between years (P=0.67). Box plots as in Fig. 1. † P<0.05 before outliers were removed.

Discussion

Defending offspring from predators is a risky behavior that varies across species and contexts (Dugatkin and Godin 1992; Lima 2009). Predator guilds are often diverse, and all predators do not pose equal threats to both adults and their young. As a result, parents that accurately assess the risks posed by different predators and scale their responses appropriately should have higher lifetime fitness (Montgomerie and Weatherhead 1988; Clutton-Brock 1991; Caro 2005). We found evidence for risk assessment and plastic antipredator behavior in nesting female house wrens. Females responded with similar levels of aggressive nest defense behavior when encountering a simulated snake or chipmunk at their nest box, both of which posed a higher risk to offspring than adults. In contrast, females responded significantly less aggressively to a simulated hawk that posed a higher risk to adults than offspring, and also they took longer to return to their nest box after encountering the hawk than the snake. This plasticity in nest defense behavior is potentially adaptive because it could maximize lifetime fitness despite the potential costs to the current clutch of reduced aggression against certain types of predators (Trivers 1974;

Montgomerie and Weatherhead 1988). Collectively, these results are consistent with the predictions of parental investment theory (Montgomerie and Weatherhead 1988; Caro 2005), and similar parental risk assessment in different contexts has been previously observed in house wrens and other species (Lima 2009; Mahr et al. 2015; Duré Ruiz et al. 2018).

We also found that female wrens responded similarly to a simulated snake at the nest box between the two breeding seasons, which indicates that the mean antipredator response is stable at the population level despite limited sampling of the same females between years. This population-level consistency is in stark contrast to the large amount of individual variation among females in how strongly they responded to each of the simulated predators. For example, the most aggressive females hit the snake and chipmunk decoys over 50 times in a seven-minute period while the least aggressive females never dove at or hit the decoys. Parental investment is predicted to be higher when clutch sizes are larger, but we found no evidence of a relationship between offspring number and nest defense behavior, and this prediction has received mixed support more broadly (Montgomerie and Weatherhead 1988; Caro 2005). One limitation of our study is that 80% of the females were defending clutches of 4-6 offspring, so it is likely that we lacked a sufficient range and samples of clutch sizes to reveal any relationship with antipredator behavior. A proper test of this relationship could involve experimental manipulations of clutch size, but there are other variables such as parent and offspring age that could also explain some of the individual variation (Caro 2005). Similar to our clutch size data, the nestling age range in this study of 2-7 days of age was insufficient to thoroughly test this hypothesis.

One important exception to the persistent among individual variation in female behavior occurred in response to the simulated hawk. Although females varied in how closely they approached and how much time they spent within five meters of the hawk, none of the 41

females ever hit the hawk or even flew over it. This consistency among female wrens indicates that when the predatory threat surpasses a certain level it is possible to see uniformity in a typically plastic behavior. Furthermore, the total absence of overt aggression towards the hawk supports the hypothesis that selection favors risk assessment as well as the prediction that selection can act strongly against behaviors that carry higher risks such as attacking a predator capable of injuring or killing an adult. However, our observations of heterospecific responses to the hawk and other simulated predators show that risk assessment is not uniform across songbird species.

Neighboring conspecifics and heterospecific songbirds frequently responded to our simulated predators by approaching and alarm calling, but very few attacked or flew directly over the decoys, including the snake and chipmunk. This observation is consistent with mobbing behavior, and the reliance on alarm calls rather than overt aggression likely reflects the reduced threat posed to these neighbors by a predator that was not in close proximity to their own offspring (Colombelli-Négrel et al. 2010; Kryštofková et al. 2011). Similar to the antipredator behavior of female wrens, the number of conspecifics and heterospecifics that responded did not differ significantly between the snake and chipmunk. In contrast, more conspecifics responded to the snake while more heterospecifics responded to the hawk in our second experiment. This result is consistent with broader avoidance of the hawk by house wrens, but it also highlights that certain heterospecific species respond in greater numbers to the hawk than the snake. Specifically, larger songbirds including tree swallows (*Tachycineta bicolor*), northern cardinals (*Cardinalis cardinalis*), and blue jays (*Cyanocitta cristata*) appeared in substantially higher numbers when the hawk was present, and tree swallows frequently dove at the hawk, which was in stark contrast with all other species. The willingness of these heterospecifics to risk mobbing

the hawk might be explained by the observation that Cooper's hawks tend to prefer larger songbirds as prey (Roth and Lima 2003; Rosenfield et al. 2020), so the hawk posed a greater risk to both adults and offspring in these species. However, the intensity of aggression from tree swallows is surprising given that their nests are concealed in cavities and more protected than the open cup nests of the northern cardinals and blue jays (Montgomerie and Weatherhead 1988).

Reactive nest defense behavior can be subdivided into (1) behaviors that distract or redirect the predator towards the signaller and (2) behaviors that involve acts of aggression towards the predator (Caro 2005; Lima 2009). Smaller organisms like house wrens may rely on distraction rather than attacking predators above a certain size because they lack the body size or armaments necessary to successfully drive the predator away (Montgomerie and Weatherhead 1988; Winkler 1992). If the likelihood of physically deterring the predator is low, then any amount of risk to the parent from attacking the predator could justify an antipredator response focused only on distraction or even no response at all. For example, when responding to human researchers that are actively removing nestlings from the nest and posing a major threat, house wrens in our population have never been observed diving at or hitting the researchers, only approaching and alarm calling. Thus, it is possible that house wrens and other organisms employ a size-based threshold process in which physical attacks are limited to predators below the size threshold and distraction methods are used regardless. Multiple species employ a similar threshold-based approach when judging predator proximity by increasing the intensity of their antipredator behavior as predators get closer to their nest or offspring (Colombelli-Négrel et al. 2010; Kryštofková et al. 2011; Mahr et al. 2015). The size-threshold hypothesis proposed above could be tested by comparing parental responses to predators of equivalent risk but varying sizes. However, it is unlikely that size thresholds universally govern antipredator behavior given that

certain species of limited size and armaments like northern mockingbirds (*Mimus polyglottos*) are notorious for attacking humans and other large predators that approach their nests (Levey et al. 2009).

Although risk assessment and plasticity in nest defense behavior appears to be common across parental organisms, including house wrens, understanding the proximate and ultimate factors that drive the individual variation within and among species continues to be challenging (Montgomerie and Weatherhead 1988; Caro 2005; Butler et al. 2009). To adequately test the determinants of nest defense, it is both important and extremely difficult in field-based systems to isolate and vary a small number of potentially important variables while controlling for all others (Regelmann and Curio 1983; Winkler 1992). We attempted to test for connections between nest defense and fitness, but we found no evidence for a relationship between nest defense behavior and clutch size or any differences in behavior between nests that fledged and those that failed. However, both comparisons were limited by small sample sizes, insufficient controls for potentially important variables such as female age, and the fact that our nests benefitted from extra protection of artificial predator guards. In addition, there is very little direct evidence to support our assumptions of the threats posed to parents that physically attack nest predators and the parent's likelihood of success at deterring predators in this and other systems (but see Samelius and Alisauskas 2006). Despite these limitations, we did observe evidence for risk assessment in how female wrens responded to different types of predators at the nest box, and their behavior matched our predictions of the relative threats posed by each predator type. Future work in this and other species should continue to focus on elucidating the causes and consequences of the large among individual variation in parental antipredator behavior.

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Supplementary Data

Table S1 – There were no detectable relationships between nestling number and the strength of the nest defense response in female house wrens.

	2020 (Snake vs. Chipmunk)		2021 (Snake vs. Hawk)	
Behavior	*t*	*P*	*t*	*P*
Hits	0.09	0.93	-0.58	0.56
Flyovers	-0.13	0.90	0.86	0.39
Time within 5 m	-1.69	0.10	1.48	0.14
Closest approach	1.52	0.13	0.08	0.93
Latency to return	-0.58	0.56	-0.15	0.88