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

Running Title: Risk assessment and nest defense in wrens

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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 despite substantial individual variation among females. However, female wrens never dove at or attacked the Cooper's hawk while they frequently attacked both the snake and chipmunk decoys. Neighboring house wrens from adjacent territories were also less likely to respond to the hawk, but more heterospecifics mobbed the hawk than the snake decoy. Collectively, these results show that risk assessment and the strength of the antipredator response varies substantially both within and among species. Female house wrens exhibit plasticity in their nest defense 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 have limited capabilities for self-defense or escape without parental assistance (e.g., Suzuki 2011). 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).

In this study, we tested for evidence of risk assessment by northern house wrens (*Troglodytes aedon aedon*) during simulated interactions with predators at their nest that posed different threats to adults and nestlings.

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 adjust the strength of their nest defense relative to risks associated with each type of predator (see below). 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 nestlings, which are still vulnerable to raptors and other avian predators despite the protection offered by an enclosed nest box (Milsap et al. 2013; 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 and lower threat posed to nestlings. Parental investment theory also predicts a positive relationship between the intensity of nest defense and the size of the reproductive investment (Montgomerie and Weatherhead 1988, Caro 2005). We tested this prediction by comparing nest defense behavior to clutch size and nestling age, which varied slightly across our predator treatments. Finally, we examined the community-level antipredator response by quantifying the number of conspecific and heterospecific individuals that responded to each predator type.

Methods

Study System and Sites

Northern house wrens (*Troglodytes aedon aedon*) 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 nestling predators is diverse and includes mammals ranging in size from mice (e.g., *Peromyscus* spp.) to bears (*Ursus spp.*), songbirds (e.g.,

139 *Cyanocitta* spp.), woodpeckers (e.g., *Melanerpes* spp.), raptors, and snakes (Johnson 2020). At
140 our study sites in Delaware and Union Counties, Ohio, USA, the most common house wren nest
141 predators are small mammals and snakes, but multiple raptor species also frequent our sites and
142 pose risks to adults and offspring (DGR, personal observation).

143 We monitored house wren breeding success at 137 nest boxes divided between six
144 locations during the 2020 and 2021 breeding seasons. The nest boxes varied slightly in
145 dimensions due to landowner preferences, but the average internal dimensions were 110 x 115 x
146 195 mm and the entrance hole size was 38 mm. Each nest box was mounted on a conduit pipe
147 approximately 1.75 m above the ground, and the pipe was covered in axle grease to limit access
148 by predators. The majority of the boxes were located in edge habitat near the transition from
149 grass or field to forest, but a small subset was located in suburban backyards (N=7).

150

151 *Banding of Adults and Nestlings*

152 We captured male house wrens using mist nets and a brief playback of conspecific song.
153 Each male was banded with a USFWS aluminum leg band and a unique combination of 2-3
154 additional colored bands for identification from a distance. We also collected morphological
155 measurements and a small blood sample for an unrelated study. Banding only males allowed us
156 to differentiate between the sexes during our behavioral trials (see below), and because most
157 females were never captured, it also minimized the likelihood that the capture process disrupted
158 the female's antipredator response. Any females that were captured accidentally (Experiment 1,
159 N = 6 of 38 females; Experiment 2, N = 10 of 38 females) were rapidly banded and immediately
160 released within a few minutes of capture to limit any effects of handling on subsequent behavior.
161 All subjects were captured and banded at least 24 hours before any behavioral trials, and most

162 females were captured more than one week before their first trial (Experiment 1, $N = 4/6$;
163 Experiment 2, $N = 5/10$).
164 We also monitored the number of eggs laid, hatching success, and offspring development
165 for every nest box. We counted the number of nestlings at the end of each behavioral trial (see
166 below), and banded nestlings when they were 8 days old. Each nestling received a single
167 USFWS aluminum band, and we collected morphological measurements and a small blood
168 sample. After banding, we passively monitored each nest box until all visible and acoustic
169 activity ceased. Unless the contents of the nest suggested otherwise (i.e., presence of dead
170 nestlings and/or their parts), we assumed that empty nests successfully fledged.
171
172 *Experiment 1: Chipmunk v. Snake Decoy*
173 In 2020, we conducted behavioral trials between June 9 and July 24 at 47 nest boxes containing
174 nestlings between the ages of 3-7 days old. A smaller age range was impossible due to the
175 distances between our sites and other field-related challenges (e.g., weather). Two life-like
176 rubber predator decoys, an eastern chipmunk (*Tamias striatus*; Safari Ltd.) and an eastern
177 ratsnake (*Pantherophis alleghaniensis*; Flybanboo), were used to elicit nest defense behavior.
178 Both of these species are commonly observed at our study sites. We waited for each female to
179 enter or land on the nest box before flushing them away during setup. One predator was placed
180 on top of the nest box, and then on the following day the other predator was presented in a
181 counterbalanced order to control for order effects. Although these stimuli are terrestrial
182 predators, both are capable of climbing nearby vegetation and approaching the nest box from
183 above, making their appearance on top of the box biologically relevant.

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. We positioned the camera in tall grass or other vegetation to minimize its disruption to the focal birds, and observers maintained a distance of roughly 15m away from the nest box. 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). Post-trial data for some of the females were lost due to technical issues or user error.

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 counted the number of hits, defined as any contact the female made with the decoy, and the number of flyovers, defined as flights by the female over the decoy. We also measured the amount of time females spent within five meters of the nest box, the amount of time that females spent physically on or in the nest box, and the female's closest approach (m) to the decoy. The five-meter threshold was chosen because all boxes had potential perches within five meters of the nest box despite variation in vegetative cover among our sites. Any female that hit the decoy was given a closest approach value of zero meters. We also noted whether or not the female produced alarm calls, whether the female's mate was present during the trial, and the male's level of aggression relative to the

207 female on an ordinal scale of less than, equal to, or greater than the female's aggression.
208 Neighboring conspecifics and heterospecifics sometimes responded to the predator decoys by
209 approaching, alarm calling, and in rare cases flying over and hitting the decoy. During each trial,
210 we noted the number of individuals of each species that approached within 5 meters of the
211 decoy. To avoid counting incidental birds that were not responding to the predator, we only
212 counted individuals that either oriented towards the decoy or produced alarm calls.
213 Behavioral data from both predator trials for an individual female were excluded from
214 our final analysis if either of the decoys were knocked off the box during either trial (*N*=3 of 47
215 total trials; 6.4%). However, if the chipmunk decoy was knocked over but remained on the box,
216 the trials were not excluded (*N*=5 of 47 trials; 10.6%). We also excluded trials if the female did
217 not return to the box within seven minutes of initiating the trial (*N*=6 of 47 trials, 12.8%). In
218 response to this limitation, we adjusted our protocol early in the season so that we removed the
219 decoy and reset the trial if the female did not return within 4 minutes after the trial was initiated.
220 Once the female returned to the box, we flushed her away, placed the predator decoy, and started
221 the seven-minute observation again. In summary, nine trials (of 47 trials; 19.1%) were excluded
222 and our final sample size was 38 paired behavioral trials.
223
224 *Experiment 2: Hawk v. Snake Decoy*
225 In 2021 between June 6 and July 30, we replicated this experiment in the same house
226 wren population, but we replaced the chipmunk decoy with a taxidermied, juvenile Cooper's
227 hawk (*Accipiter cooperii*), which is commonly observed at our study sites. Although Cooper's
228 hawks primarily forage on small or medium-sized adult songbirds and mammals, they have been
229 frequently observed capturing nestlings from both cavity nests in bird boxes and open-cup nests

in the forest canopy (Milsap et al. 2013, Rosenfield et al. 2020). 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. The snake decoy was knocked off the nest box during three trials (of 41 total; 7.3%), and we excluded both the snake and hawk trials from those subjects in our final analysis for a total sample of 38 paired behavioral trials.

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, including six females banded in previous breeding seasons. 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.

Note on Experimental Design

Our decision to split this study into two separate experiments rather than one experiment including all three predator stimuli was driven by uncertainty stemming from the global Covid-19 pandemic. This event shutdown numerous research labs in 2020 and disruptions continued into 2021. The potential for an unpredictable and abbreviated 2020 field season led us to err on the side of a less complicated protocol to maximize our sample size. We chose to focus on two nest predators (snake, chipmunk) in 2020, and then based on the results of that experiment, we transitioned to one nest predator (snake) and one predator of nests and adults (hawk) in 2021.

Statistical analysis

For each experiment, 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 nest box 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 experiments. 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. Alarm calls were recorded as a categorical variable (presence/absence), and we compared the proportion of females that produced calls between predator types for each experiment using a two-proportion z-test.

We analyzed the males' response relative to their female mate's response in both experiments using separate linear mixed models. Nests were excluded from the analysis if the

276 male was not present for both predator trials (*N*=6 in both 2020 and 2021). The linear mixed
277 models included male behavior as the dependent variable, predator type, treatment order and
278 their interaction as fixed effects, and day of the year and female ID as random effects. We also
279 compared the proportion of males that responded to each predator type in each experiment using
280 a two-proportion z-test.

281 We used a similar linear mixed model approach to determine whether the number of
282 neighboring conspecific and heterospecific individuals and the number of heterospecific species
283 that responded to the trials differed between predator types in both years. The total number of
284 conspecifics, total number of heterospecifics, and the number of heterospecific species were used
285 as dependent variables in three separate models. We included predator type, treatment order and
286 their interaction as fixed effects and day of the year and female ID as random effects.

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

295 We used linear regressions (Base R package) to test for relationships between female
296 antipredator behaviors and both clutch size and nestling age. To expand our sample size, we also
297 pooled female responses to the snake decoy across years and ran linear regressions between
298 behavior and both clutch size and nestling age. In addition, we used generalized linear mixed

299 models to test whether female antipredator behaviors predicted whether nests fledged or failed.
300 Nest outcome was included as the dependent variable with female behavior as a fixed effect and
301 day of the year and female ID as random effects. These models assumed a binomial distribution
302 to account for nest outcome being scored as a categorical variable (fledge/fail).

303 To evaluate model assumptions across our analyses, we tested for normality of model
304 residuals using Shapiro-Wilk tests and for homoscedasticity using Levene's test. As is often the
305 case with behavioral data, few of our models' residuals achieved normality and most were
306 slightly skewed towards one end of the distribution. Homoscedasticity was met for all models in
307 experiment 1 and the interannual comparisons, but only for the female return time data in
308 experiment 2. This violation was seemingly due to the uniformly weak responses elicited by the
309 hawk decoy in experiment 2. An abundance of evidence has indicated that linear mixed models
310 are robust to violations of these assumptions, except in cases where the sample sizes are
311 prohibitively small or the distributions are bimodal, neither of which occurs in our data
312 (Schielzeth et al. 2020; Knief and Forstmeier 2021; and further citations within).
313

314 *Ethical Note*

315 All procedures were reviewed and approved by the Ohio Wesleyan University
316 Institutional Animal Care and Use Committee (Protocol Numbers: Spring_2020-21-01_Reichard
317 and 05-2021-06), U.S. Federal Bird Banding Lab (Permit Number: 24035), and State of Ohio
318 Department of Natural Resources (Permit Numbers: 23-009, 23-010). To minimize stress in the
319 free-living birds used in this study, we attempted to capture and mark only the male of each pair,
320 which was the minimum required to facilitate individual identification during our behavioral
321 trials. All birds were immediately removed from mist nets after capture, processed rapidly to

limit handling time, and then safely released at the capture site. No adverse effects were observed from these widely established techniques. Although our behavioral trials involved the observation of a simulated predator at the nest box, all subjects returned to normal parental care after the completion of the trial and no nests were abandoned as a result of these experiments.

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<0.001, *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). There was also no difference in the number of females (36 of 38 total for both predators; 94.7%) that produced alarm calls in response to each predator (χ^2_1<0.001, *P*>0.99). 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; Table S1, Supplementary Materials) or significant interactions between treatment order and predator type (P>0.09 in all cases; Table S1). The only exception was a trend towards an interaction between predator types and order for the number of hits (*P*=0.09). Female wrens tended to hit the chipmunk decoy more when it was presented during the first trial, but they tended to hit the snake decoy more when it was presented during the second trial.

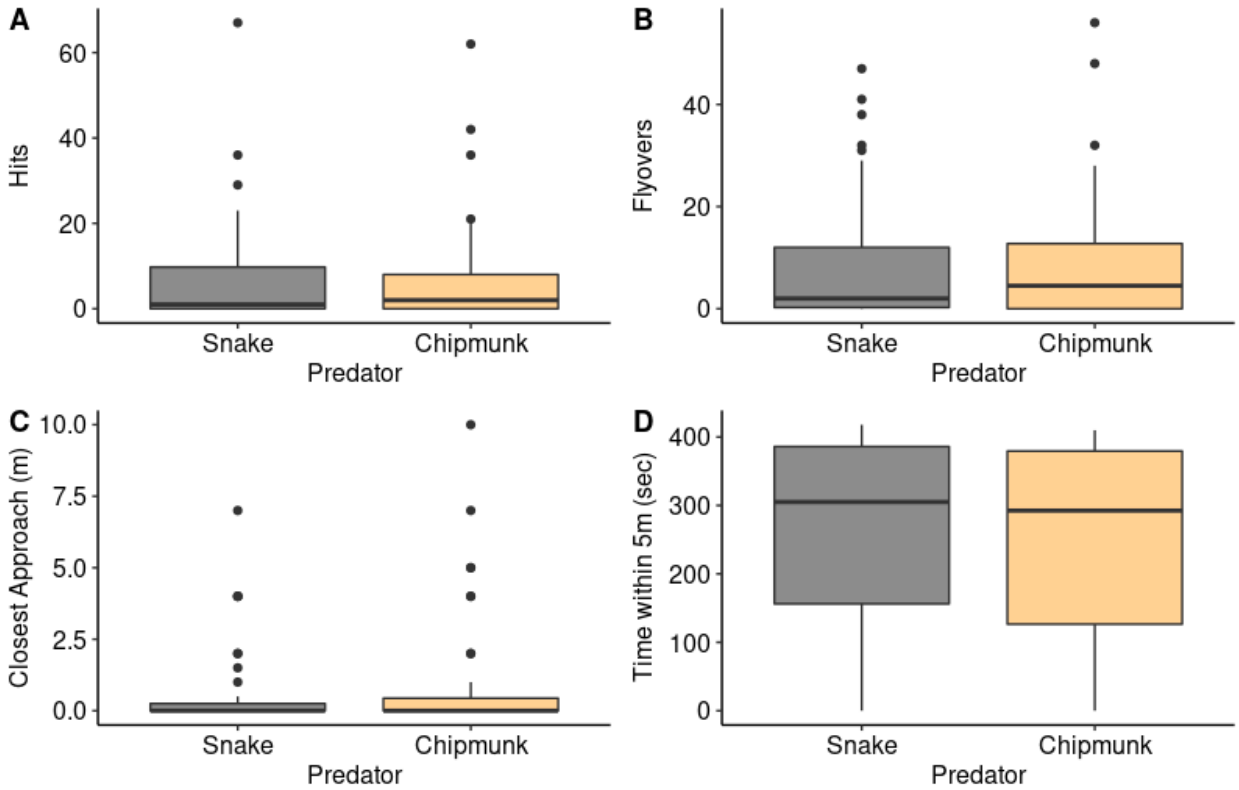

Figure 1 — Responses of female house wrens to a snake or chipmunk decoy on top of their nest box during a seven-minute trial. There were no significant differences in the number of (A) hits (P=0.91), (B) flyovers (P>0.99), (C) how closely females approached each model (P=0.23), or (D) how much time females 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.

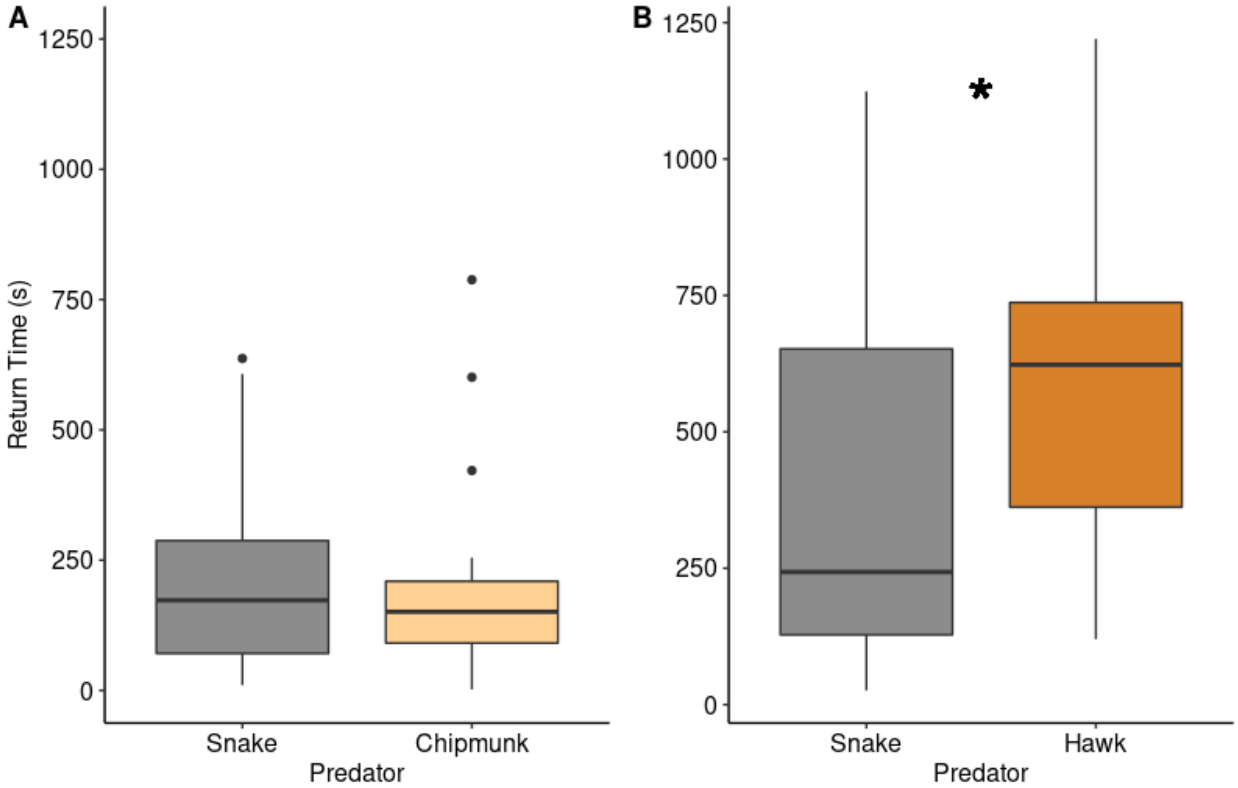

*Figure 2 — Female house wrens modulate how quickly they resume normal behavior after encountering different nest predator decoys. (A) There was no detectable difference in how long it took female wrens to resume normal behavior after encountering a snake decoy versus a chipmunk decoy (P=0.81). (B) Females 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.*

Males were present for 87% of snake trials (*N*=33 of 38 total) and 92% of chipmunk trials (*N*=35 of 38), but this difference was not statistically significant (χ^2_1=0.14, *P*=0.71). There was no detectable difference between males' aggressiveness relative to the female's aggression toward the snake decoy versus the chipmunk (*N*=32, χ^2_1=0.67, *P*=0.41). There were also no

365 detectable effects of order on male aggressiveness (χ^2_1=2.05, *P*=0.15), nor an interaction between
366 predator and order (χ^2_1=0.23, *P*=0.63).
367 The number of conspecific (χ^2_1=0.28, *P*=0.59) and heterospecific individuals (χ^2_1=0.49,
368 *P*=0.48) and the number of heterospecific species (χ^2_1=0.06, *P*=0.81) that responded to the
369 predator decoys did not differ detectably between the two predator types. We also found no
370 significant effects of treatment order on any of these measurements (*P*>0.08 in all cases, Table
371 S2), though we observed that more heterospecific species tended to mob the predator decoy
372 presented during the first trial regardless of predator type (*P*=0.08). We did note there was a
373 significant predator type by treatment order interaction on the number of heterospecific
374 individuals that responded to the predator decoys (χ^2_1=4.23, *P*=0.04). More heterospecifics
375 responded to the snake when it was presented during the first trial, but there was a stronger
376 heterospecific response to the chipmunk during the second trial.
377 We found no relationship between clutch size and the number of hits, number of flyovers,
378 time spent within 5 meters of the nest box, closest approach, or latency for female wrens to
379 return to the nest box after the trial (*P*>0.10 in all cases; Table S3). Similarly, there were no
380 significant relationships between any of the female behaviors and nestling age (*P*>0.40 in all
381 cases; Table S4). We also found that none of the nest defense behaviors were predictive of
382 whether nests fledged (*N*=21) or failed (*N*=4; *P*>0.42 for all behaviors; Table S5). The sample
383 size for this comparison is reduced (*N*=25) because multiple nests remained active after the end
384 of our data collection and their end point is unknown.
385
386 *Experiment 2: Hawk v. Snake Decoy*

387 Similar to Experiment 1, female wrens frequently performed hits and flyovers while
388 defending against the snake decoy, with 25 out of 41 females (61.0%) hitting the snake model at
389 least once and 38 out of 41 females (92.7%) flying over at least once. Notably, none of the
390 female wrens performed hits or flyovers while defending against the hawk decoy (Figure 3).
391 Statistically, females performed significantly more hits (χ^2_1=14.72, $P < 0.001$) and flyovers
392 (χ^2_1=41.20, $P < 0.001$) in response to the snake decoy (Figure 3A-B). Females approached
393 significantly closer to the snake (χ^2_1=116.9, $P < 0.001$; Figure 3C), but the amount of time spent
394 within 5 meters was not significantly different (χ^2_1=3.04, P=0.08) despite a marginal trend
395 towards more time in close proximity to the snake (Figure 3D). We found no evidence for an
396 effect of treatment order (P>0.15 in all cases; Table S6) or a predator type by treatment order
397 interaction (P>0.06 in all cases) for total hits, total flyovers, or closest approach, but there was a
398 trend towards an order effect for time spent within 5 meters of the predator decoy (χ^2_1=3.48,
399 P=0.06; Table S6). Female wrens tended to spend more time within 5 meters of the decoy during
400 the second trial than during the first trial regardless of the order in which predators were
401 presented. An equal number of females (34 of 38 total; 89.5%) produced alarm calls in response
402 to each of the two predator decoys (χ^2_1<0.001, P>0.99). Finally, after the removal of the predator
403 decoys, female wrens took significantly more time to re-enter the nest box after the hawk trials
404 than after the snake trials (χ^2_1=4.80, P=0.03; Figure 2B). There was no detectable effect of
405 treatment order (χ^2_1=0.02, P=0.89) or an interaction between order and predator type (χ^2_1=0.07,
406 P=0.79) for this behavior.
407 Males were present for 87% of snake trials (N=33 of 38 total) and 95% of hawk trials
408 (N=36 of 38), which was not statistically different (χ^2_1=0.63, P=0.43). There was a significant
409 difference in the strength of the males' response relative to the females' response between the

two predator types (N=32, χ^2_1=5.20, P=0.02). When responding to the hawk, 75% (25 of 33 total) of males responded as aggressively as their female mate, and none of the males hit or flew over the hawk decoy. However, the relative male response to the snake was more variable with only 33% (11 of 33) males responding equally relative to their mate and 39% (13 of 33) responding more aggressively than their mate. Thus, on average, males responded more aggressively to the snake than their mates, but equally aggressively to the hawk. As in 2020, there was no detectable order effect (χ^2_1=0.10, P=0.75) or predator by order interaction (χ^2_1=<0.001, P>0.99) on male behavior.

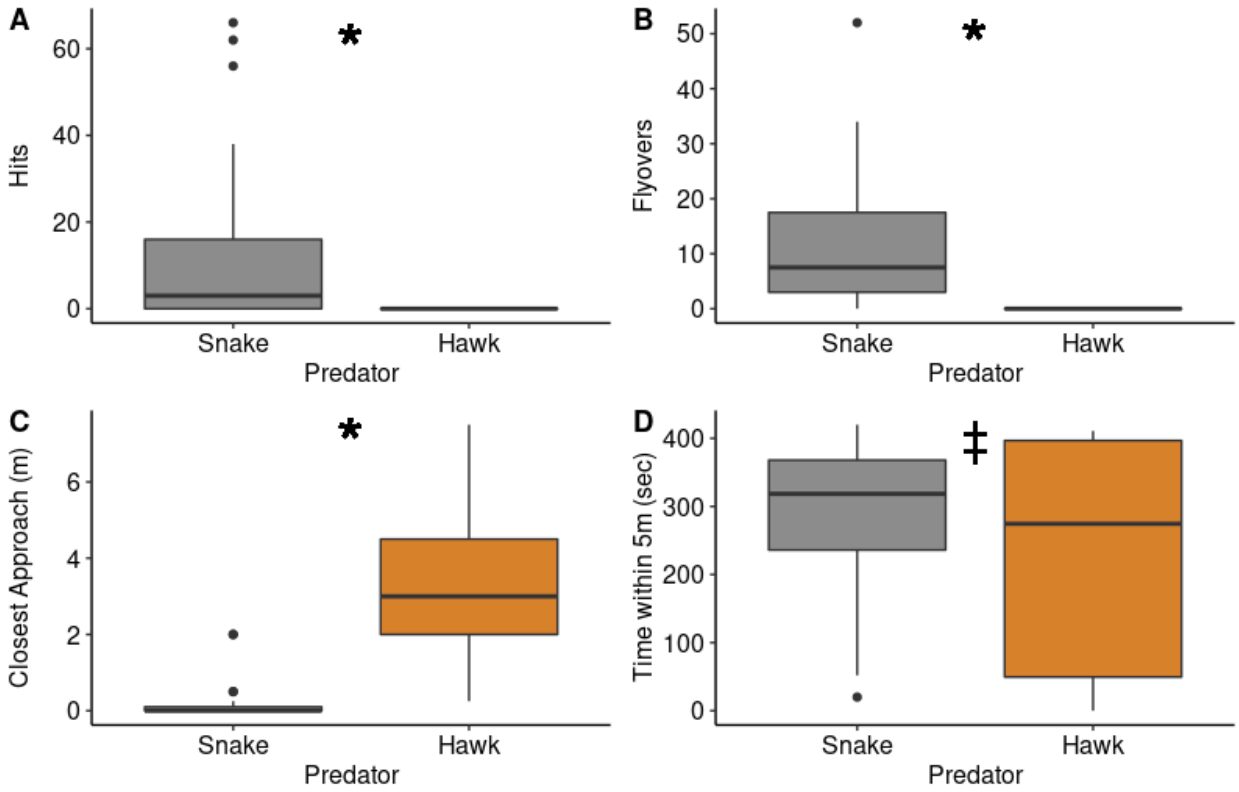

Figure 3 — Responses of female house wrens to a snake or hawk decoy on top of their nest box during a seven-minute trial. Female house wrens performed significantly more (A) hits (P<0.001) and (B) flyovers (P<0.001) against the snake decoy than against the hawk decoy.

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; Table S7) or a predator type by treatment order interaction (*P*>0.19 in all cases) for heterospecific and conspecific responses to the two decoys.

As in 2020, we found no evidence of a relationship between clutch size (*P*>0.14 in all cases; Table S3) or nestling age (*P*>0.63 in all cases; Table S4) and any female nest defense behaviors. 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

446 years (Figure 4). We found a significant difference (χ^2_1=4.28, *P*=0.04) between the female
447 wrens' closest approach to the snake decoy in 2020 ($\bar{x}$=0.72 meters) versus in 2021 ($\bar{x}$=0.18
448 meters; Figure 4). However, after removing two low-responding outliers from the 2020 data set
449 (closest approach = 7 m, 4 m respectively), the difference was no longer significant (χ^2_1=2.36,
450 *P*=0.13; Figure 4). A slightly higher proportion of females (36 of 38; 94.7%) produced alarm
451 calls in 2020 than in 2021 (34 of 38; 89.5%), but these proportions were not statistically different
452 (χ^2_1=0.18, *P*=0.67). We did not detect a difference in the strength of the male response relative to
453 the female between years (χ^2_1=2.80, *P*=0.09). However, there was a trend suggesting that males
454 were more aggressive relative to females in 2020 than in 2021.
455 Because the majority of the female behaviors were not statistically different between the
456 two years, we combined both years of snake response data to increase our sample size and test
457 for a relationship between both clutch size and nestling age and nest defense behavior. However,
458 the results remained unchanged as we found no evidence for any significant relationships
459 between female behavior and clutch size (*P*>0.33 for all behaviors; Table S8) or nestling age
460 (*P*>0.09 for all behaviors; Table S9). The only potential exception was a slight trend towards
461 females spending less time within 5 m of the snake decoy as nestling age increased (*P*=0.09;
462 Table S4).
463

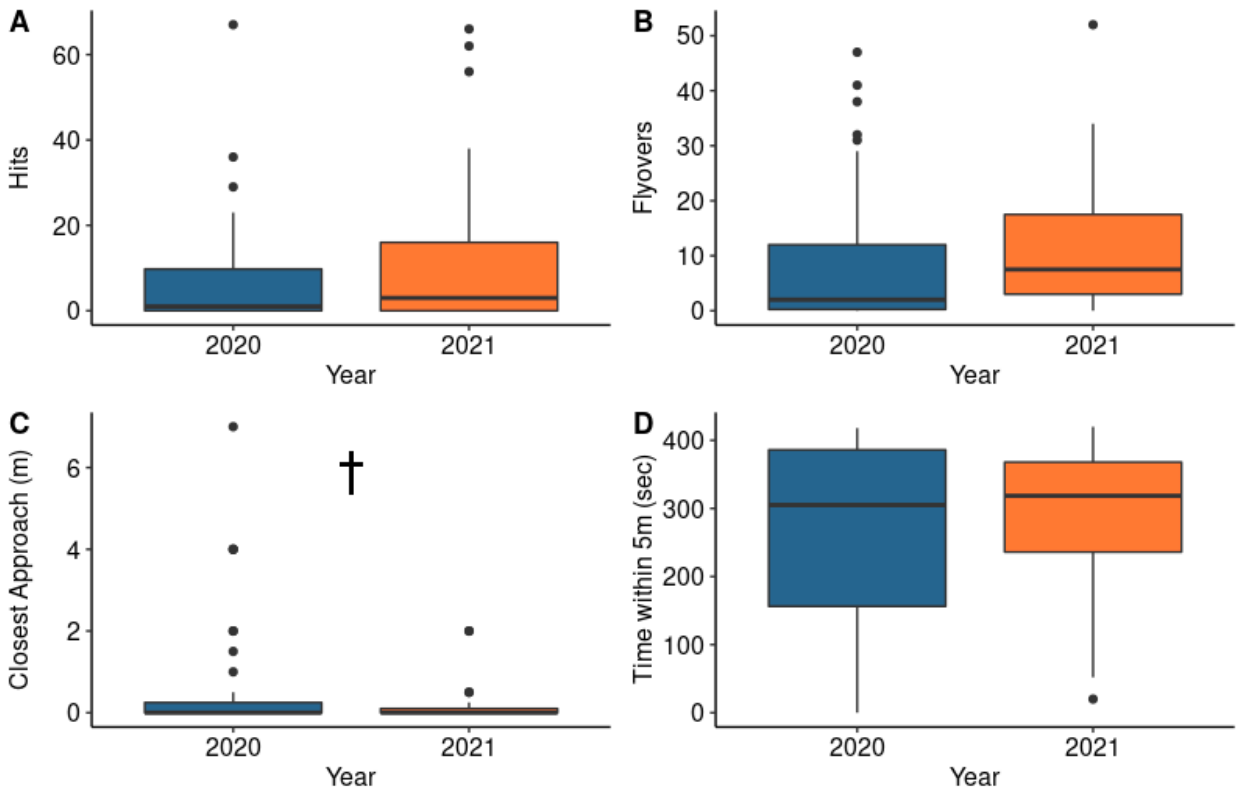

Figure 4 — Interannual comparison of female house wren nest defense behaviors in response to a snake decoy across two breeding seasons. There were no significant differences in (A) the number of hits (P=0.30) or (B) the number of flyovers (P=0.30). (C) Female wrens made significantly closer approaches 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 from 2020 data.*

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 house wrens. Both female and male wrens 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, the wrens responded significantly less aggressively to a simulated hawk that posed a higher risk to adults than nestlings, and females also 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 parents that avoid high risk predators like the hawk are more likely to live longer and have higher lifetime fitness despite the potential costs to the current clutch of offspring (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. Male aggression toward the snake and chipmunk decoys also showed substantial individual variation. Although males and females frequently responded to the decoys with different levels of intensity, neither sex was consistently more aggressive than the other, supporting earlier work that has shown nest defense to be an effort shared by both parents (Fernández et al. 2015; Fernández and Carro 2022).

Parental investment is predicted to be higher when clutch sizes are larger and offspring are older. However, we found no evidence of a relationship between offspring number or age and nest defense behavior, and both of these predictions have received mixed support more broadly (Montgomerie and Weatherhead 1988; Caro 2005). One limitation of our study is that we may have lacked a sufficient range and samples of clutch sizes and nestling ages to reveal any relationships with antipredator behavior. For example, 80% of the wren pairs in our study were defending clutches of 4-6 offspring, and 87% had nestlings between 3-6 days post hatch. A proper test of these relationships could involve experimental manipulations of clutch size or sampling nest defense at two distant points in the nestling phase such as soon after hatch and again near fledging. Additionally, there are other variables such as parental age and experience that could explain some of the individual variation in nest defense behavior (Caro 2005).

Two important exceptions to the persistent variation among individuals in nest defense behavior occurred with the production of alarm calls across the predator stimuli and also in the behavioral responses to the simulated hawk. Females almost universally produced alarm calls in response to all three of our predator stimuli, and although it was not directly measured, males

523 also called frequently regardless of the predator type. Alarm calls are a lower risk behavior than
524 the other nest defense behaviors sampled here, which might explain their consistent production.
525 When produced near the nest, alarm calls can serve many adaptive functions including
526 distracting the predator (Fasanella and Fernández 2009), inducing neighboring birds to mob the
527 predator (Templeton et al. 2005), and alerting the young inside the nest that a predator is nearby
528 (Suzuki 2011). Previous research in another house wren population found that parents produced
529 alarm calls even in the presence of a non-predatory heterospecific bird at their nest (Fasanella
530 and Fernández 2009), suggesting that alarm calls might function as a general response to any
531 disturbance at the nest box. However, wren parents also produced more alarm calls in response
532 to a potential predator rather than a non-predatory control bird (Fasanella and Fernández 2009)
533 and more alarm calls were produced as a simulated snake moved closer to the nest (Fernández
534 and Carro 2022). Although we did not measure whether the quantity of alarm calls differed
535 between predator types in our study, it seems clear that alarm calls are a consistent and important
536 component of the antipredator response, and the intensity of these calls might signal information
537 about the predator type and level of risk posed to the nestlings (Fasanella and Fernández 2009,
538 Fernández and Carro 2022).

539 When responding to the hawk decoy, females varied in how closely they approached and
540 how much time they spent within five meters of the hawk, but none of the 41 females ever hit the
541 hawk or even flew over it. Additionally, none of the males hit or flew over the hawk decoy. This
542 consistency among individuals and between the sexes indicates that when the predatory threat
543 surpasses a certain level it is possible to see uniformity in a typically plastic behavior.
544 Furthermore, the total absence of overt aggression towards the hawk supports the hypothesis that
545 selection favors risk assessment as well as the prediction that selection can act strongly against

546 behaviors that carry higher risks such as attacking a predator capable of injuring or killing an
547 adult. However, our observations of heterospecific responses to the hawk and other simulated
548 predators show that risk assessment is not uniform across songbird species.

549 Neighboring conspecifics and heterospecific songbirds frequently responded to our
550 simulated predators by approaching and alarm calling, but very few attacked or flew directly
551 over the decoys, including the snake and chipmunk. This observation is consistent with mobbing
552 behavior, and the reliance on alarm calls rather than overt aggression likely reflects the reduced
553 threat posed to these neighbors by a predator that was not in close proximity to their own
554 offspring (Colombelli-Négrel et al. 2010; Kryštofková et al. 2011). Similar to the antipredator
555 behavior of the wren parents, the number of conspecifics and heterospecifics that responded did
556 not differ significantly between the snake and chipmunk. In contrast, more conspecifics
557 responded to the snake while more heterospecifics responded to the hawk in our second
558 experiment. This result is consistent with broader avoidance of the hawk by house wrens,
559 particularly when the hawk is not posing a direct threat to their own offspring. It also highlights
560 that certain heterospecific species actually respond in greater numbers to the hawk than the
561 snake. Specifically, larger songbirds including tree swallows (*Tachycineta bicolor*), northern
562 cardinals (*Cardinalis cardinalis*), and blue jays (*Cyanocitta cristata*) appeared in substantially
563 higher numbers when the hawk was present, and tree swallows frequently dove at the hawk,
564 which was in stark contrast with all other species. The willingness of these heterospecifics to risk
565 mobbing the hawk might be explained by the observation that Cooper's hawks tend to prefer
566 larger songbirds as prey (Roth and Lima 2003; Rosenfield et al. 2020), so the hawk posed a
567 greater risk to both adults and offspring in these species. However, the intensity of aggression
568 from tree swallows is surprising given that their nests are concealed in cavities and more

569 protected than the open cup nests of the northern cardinals and blue jays (Montgomerie and
570 Weatherhead 1988).

571 Reactive nest defense behavior can be subdivided into (1) behaviors that distract or
572 redirect the predator towards the signaler and (2) behaviors that involve acts of aggression
573 towards the predator (Caro 2005; Lima 2009). Smaller organisms like house wrens may rely on
574 distraction rather than attacking predators above a certain size because they lack the body size or
575 armaments necessary to successfully drive the predator away (Montgomerie and Weatherhead
576 1988; Winkler 1992). If the likelihood of physically deterring the predator is low, then any
577 amount of risk to the parent from attacking the predator could justify a distraction-based
578 antipredator response or even no response at all. For example, when responding to human
579 researchers that are actively removing nestlings from the nest and posing a major threat, house
580 wrens in our population have never been observed diving at or hitting the researchers, only
581 approaching and alarm calling. Thus, it is possible that house wrens and other organisms employ
582 a size-based threshold process in which physical attacks are limited to predators below the size
583 threshold and distraction methods are used regardless. Multiple species employ a similar
584 threshold-based approach when judging predator proximity by increasing the intensity of their
585 antipredator behavior as predators get closer to their nest or offspring (Colombelli-Négrel et al.
586 2010; Kryštofková et al. 2011; Mahr et al. 2015). The size-threshold hypothesis proposed above
587 could be tested by comparing parental responses to predators of equivalent risk but varying sizes.
588 However, it is unlikely that size thresholds universally govern antipredator behavior given that
589 certain species of limited size and armaments like northern mockingbirds (*Mimus polyglottos*)
590 are notorious for attacking humans and other large predators that approach their nests (Levey et
591 al. 2009).

592 Although risk assessment and plasticity in nest defense behavior appears to be common
593 among organisms with altricial offspring, including house wrens, understanding the proximate
594 and ultimate factors that drive the individual variation within and among species continues to be
595 challenging (Montgomerie and Weatherhead 1988; Caro 2005; Butler et al. 2009). To adequately
596 test the determinants of nest defense, it is both important and extremely difficult in field-based
597 systems to isolate and vary a small number of potentially important variables while controlling
598 for all others (Regelmann and Curio 1983; Winkler 1992). We tested for connections between
599 nest defense and fitness, but we found no evidence for a relationship between nest defense
600 behavior and clutch size or nestling age or any differences in behavior between nests that fledged
601 and those that failed. However, each of those comparisons was limited by small sample sizes,
602 insufficient controls for potentially important variables such as female age, and the fact that our
603 nests benefitted from extra protection of artificial predator guards.
604 Despite these limitations, we did observe evidence for risk assessment in how female
605 wrens responded to different types of predators at the nest box, and their behavior matched our
606 predictions of the relative threats posed by each predator type. Furthermore, we observed
607 differences in the community-level response as heterospecific songbirds responded in greater
608 numbers to the hawk than conspecifics from neighboring territories. These results indicate that
609 risk assessment is not consistent among species, and species like house wrens that respond
610 minimally to certain predators can gain some protection from the antipredator behavior of nearby
611 heterospecifics. Future work should continue to focus on elucidating the causes and
612 consequences of persistent intra- and interspecific variation in parental antipredator behavior.
613
614

615 **Data Availability Statement**

616 The data collected and analyzed to support these findings are freely available in the Mendeley
617 Data Repository at DOI:10.17632/zjb5ynz5n4.1.

618

619 **Acknowledgements**

620 We thank Ohio Wesleyan University, the Big Walnut School System, and the Davis, Koban, and
621 Fink families for access to facilities and field sites. We thank Holly Keating for analyzing
622 videos, Tamaki Yuri for preparing the taxidermied Cooper's Hawk, Lily Bonar for field
623 assistance, and Chandler Carr for measuring our nest boxes. This project was funded by the Ohio
624 Wesleyan University Summer Science Research Program.

625

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753 **Supplementary Materials**

754 *Table S1 – Analysis of predator order effects and interactions between predator type and order*
755 *for Experiment 1. Although no results were significant, we noted a trend towards an interaction*
756 *between predator type and order. Female wrens tended to hit the chipmunk decoy more when it*
757 *was presented during the first trial, but they tended to hit the snake decoy more when it was*
758 *presented during the second trial. ‡P<0.10.*

	Order effects		Interaction effects	
Behavior	χ^2	*P*	χ^2	*P*
Hits	0.02	0.89	2.84	0.09‡
Flyovers	0.02	0.89	0.95	0.33
Time within 5 m	0.00	>0.99	2.46	0.12
Closest approach	1.05	0.31	1.06	0.30
Latency to return	2.13	0.14	0.01	0.94

759

760 *Table S2 – Analysis of heterospecific and conspecific behavior for Experiment 1. Though not*
761 *significant, more species tended to mob the predator decoy during the first trial regardless of the*
762 *order in which predators were presented. There was a significant trend showing that more*
763 *heterospecific birds mobbed the snake decoy when it was presented during the first trial, but*
764 *more heterospecifics mobbed the chipmunk decoy when it was presented second ‡P<0.10,*
765 **P<0.05.*

	Order effects		Interaction effects	
Heterospecific & conspecific response	χ^2	*P*	χ^2	*P*
Heterospecific abundance	0.23	0.63	4.23	0.04*
Heterospecific richness	3.03	0.08‡	2.16	0.14
Conspecific abundance	0.21	0.65	1.14	0.29

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Table S3 – There were no detectable relationships between clutch size and nest defense behaviors by female house wrens in either experiment.

	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

Table S4 – There were no detectable relationships between nestling age and nest defense behaviors by female house wrens in either experiment.

	2020 (Snake vs. Chipmunk)		2021 (Snake vs. Hawk)	
Behavior	t	P	t	P
Hits	0.84	0.40	-0.27	0.79
Flyovers	-0.02	0.98	-0.15	0.88
Time within 5 m	0.10	0.93	-0.27	0.79
Closest approach	0.72	0.48	-0.10	0.93
Latency to return	0.04	0.97	0.48	0.63

Table S5 – Female nest defense behavior in Experiment 1 did not predict nest success.

Behavior	*z*	*P*
Hits	-0.53	0.60
Flyovers	0.83	0.41
Time within 5 m	0.95	0.34
Closest approach	0.42	0.68
Latency to return	-0.35	0.73

Table S6 – Analysis of predator order effects and interactions between predator type and order for Experiment 2. Although no results were significant, we noted a trend towards female wrens spending more time within 5 m of the decoy presented during the second trial. ‡P<0.10.

	Order effects		Interaction effects	
Behavior	χ^2	*P*	χ^2	*P*
Hits	1.12	0.29	1.12	0.29
Flyovers	1.99	0.16	1.99	0.16
Time within 5 m	3.47	0.06‡	0.74	0.39
Closest approach	2.09	0.15	1.47	0.23
Latency to return	0.54	0.46	0.87	0.35

Table S7 – Analysis of heterospecific and conspecific mobbing behavior for Experiment 2. There was no detectable order effect or interaction between predator type and order.

	Order effects		Interaction effects	
Heterospecific & conspecific response	χ^2	*P*	χ^2	*P*
Heterospecific abundance	1.30	0.25	0.01	0.94
Heterospecific richness	<0.01	0.96	1.73	0.19
Conspecific abundance	0.28	0.60	0.99	0.32

Table S8 – Focusing only on female responses to the snake decoy and pooling samples from both experiments, we again found no detectable relationships between clutch size and the strength of the nest defense response in house wrens.

Behavior	*t*	*P*
Hits	-0.40	0.69
Flyovers	0.99	0.33
Time within 5 m	0.26	0.80
Closest approach	-0.31	0.76
Latency to return	-0.49	0.63

Table S9 – Focusing only on female responses to the snake decoy and pooling samples from both experiments, we again found no detectable relationships between nestling age and the strength of the nest defense response in house wrens. However, there was a trend towards females spending less time within 5 m of the snake decoy as nestlings aged. ‡*P<0.10.*

Behavior	*t*	*P*
Hits	0.01	0.99
Flyovers	-0.77	0.45
Time within 5 m	-1.70	0.09‡
Closest approach	1.20	0.23
Latency to return	0.25	0.80