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Perceived Predation Risk Reduces the Number of Offspring Songbirds Produce per Year

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cultivation). The data indicated that CBG and capacity-building respondents were more varied in their assessments as to whether these attributes were improving or declining, but control respondents more uniformly indicated "no change." This difference is what led to statistical significance. We interpret this to illustrate that CBG and capacity-building members were in a more dynamic position of change than control members. The control members were more static, but this was in relation to a low base in terms of cattle numbers and minimal cash-crop cultivation to begin with. Unlike Liben, treatment effects for attributes such as human health or interest in children's education were either weaker or lacking at Moyale. The data indicated that a majority of respondents from all treatments perceived improvements in access to health care and heightened interest in children's education. This may reflect a higher level of public-service awareness among Moyale residents compared with that for Liben; Moyale town has been a commercial and administrative hub for a much longer time than has Negele town (in Liben).

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12. Out of 144 reasons given by 102 CBG or capacity-building respondents as to why hunger had decreased or not gotten worse, 54% were related to the use of increased income, savings, or other aspects of collective action to acquire food. The second most common reason (15%) was receiving food aid, and this was in Moyale. Livestock trade or livestock production was mentioned in only 3% of reasons given.
13. Higher personal incomes and improved extension outreach by government were commonly implicated by members of CBG and capacity-building treatments in promoting the purchase of inputs to support animal health, human health, cash-crop production, and innovative forage-management systems. Capacity building has fostered more connectivity between local people and development agencies in Liben.
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Perceived Predation Risk Reduces the Number of Offspring Songbirds Produce per Year

Liana Y. Zanette,[1]* Aija F. White,[1] Marek C. Allen,[1] Michael Clinchy[2]

Predator effects on prey demography have traditionally been ascribed solely to direct killing in studies of population ecology and wildlife management. Predators also affect the prey's perception of predation risk, but this has not been thought to meaningfully affect prey demography. We isolated the effects of perceived predation risk in a free-living population of song sparrows by actively eliminating direct predation and used playbacks of predator calls and sounds to manipulate perceived risk. We found that the perception of predation risk alone reduced the number of offspring produced per year by 40%. Our results suggest that the perception of predation risk is itself powerful enough to affect wildlife population dynamics, and should thus be given greater consideration in vertebrate conservation and management.

Predator effects on prey demography have traditionally been ascribed solely to direct killing in studies of population ecology and wildlife management, because the effect of direct killing on prey numbers can be directly observed (*1*–*3*). An emerging alternative is that the effect of predators on prey numbers may be far greater than what can be attributed to direct killing alone, if the costs of antipredator responses reduce prey reproduction and increase deaths from other causes (*1*–*7*). Antipredator responses may include changes in habitat use, vigilance, and foraging behavior (*1*, *2*, *4*–*9*), or physiological changes (*2*, *3*, *10*, *11*), any or all of which could conceivably affect prey demography. Although long suggested by theory [e.g., (*12*, *13*)], this alternative remains rarely considered in vertebrate conservation and management because of a lack of direct experimental evidence that the perception of predation risk alone is powerful enough to affect the population growth rate of free-living wildlife (*1*–*4*, *8*). The population growth rate is determined by the number of offspring produced per year in addition to juvenile and adult survival, and the number of offspring produced per year is a function of the number of propagules (eggs or neonates) and their survival to the juvenile stage (*14*). Unless direct predation can be precluded as a possible cause of death, testing whether predators can affect prey survival independent of direct killing is not possible (*1*–*3*). Because eliminating direct predation for this purpose has proven very difficult, the effect of perceived predation risk on the survival of free-living wildlife remains experimentally untested, and only one previous experiment (*6*) has documented an effect on the number of propagules (*1*–*4*, *8*).

Here, we report a 40% reduction in the number of offspring produced per year by wild, free-living female song sparrows (*Melospiza melodia*) in response to a field experiment in which we actively eliminated direct predation and manipulated perceived predation risk throughout an

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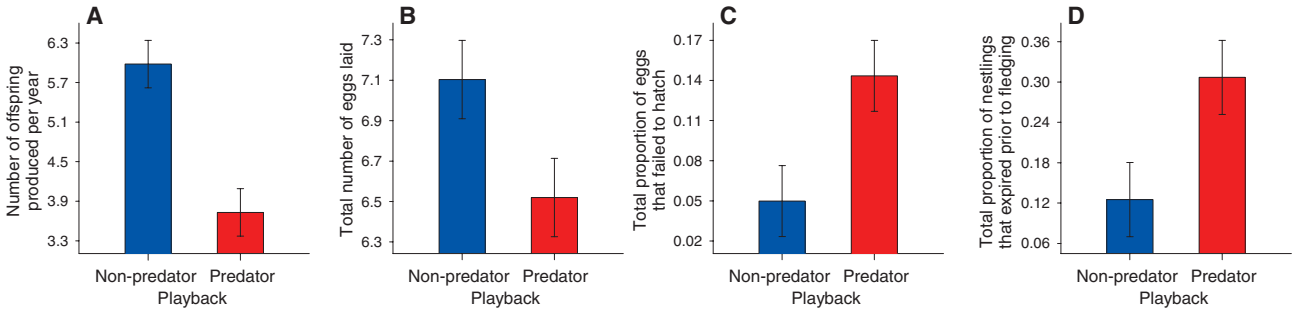


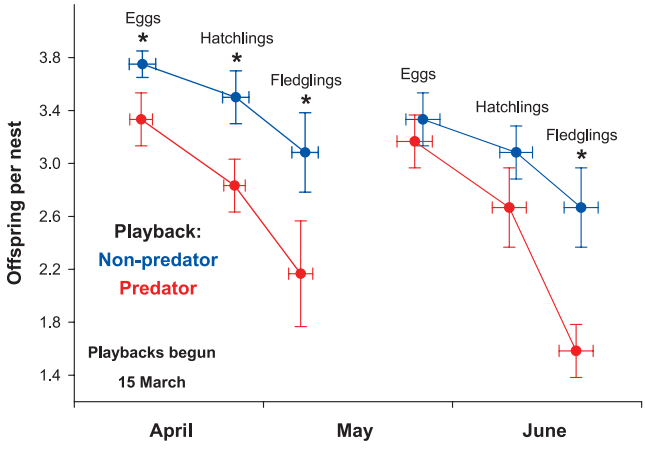
Fig. 1. Effect of perceived predation risk on offspring number among females exposed to predator or non-predator playbacks. (**A**) Number of offspring produced per year ($F_{1,22}$ = 19.69, P < 0.001). (**B**) Total number of eggs laid ($F_{1,22}$ = 4.86, P = 0.038). (**C**) Total proportion of eggs that failed to hatch ($F_{1,22}$ = 6.21, P = 0.021). (**D**) Total proportion of nestlings that expired ($F_{1,22}$ = 4.86, P = 0.038). Values are means ± SE.

entire breeding season (*15*). The song sparrow is a typical open-cup, shrub-nesting songbird, well studied by ourselves and others. We studied song sparrows on several of the small Gulf Islands (British Columbia, Canada), where they are resident year-round, generally lay at least two clutches per year, and lose at least half of their offspring to direct predation (*15*).

We actively eliminated direct predation by protecting every nest in the experiment with both electric fencing and seine netting (*15*). That we successfully eliminated direct predation was verified via continuous video surveillance over the complete nesting cycle, which permitted us to know the fate of every egg and nestling with certainty (*15*). Successfully eliminating direct predation enabled us to isolate, and so experimentally test, whether perceived predation risk could alone affect the number of offspring produced per year (*1*–*3*). To manipulate perceived risk, we composed separate playlists of the calls and sounds of either predators (raccoon, corvid, hawk, owl, cowbird) or non-predators [e.g., seal, goose, flicker, loon, hummingbird (*15*)]. Each predator was matched with a similar-sounding non-predator (e.g., raven caw with goose honk), and analyses verified that there were no significant differences in overall frequency characteristics between the two treatments (*15*). Neither were there treatment differences in the distance between speakers and nests, or in the incidence of naturally occurring predator calls and sounds (*15*).

Playbacks were broadcast every few minutes, 24 hours per day on a 4-day-on–4-day-off cycle, throughout the 130-day breeding season, over a cumulative area of 16 ha (*15*). Territories were censused beginning on 1 March 2010. We recorded all the breeding activities of each territorial female (n = 12 per treatment) throughout the entire season. We balanced the distribution of treatments across islands as much as possible (fig. S1) and included study location and female identity as random effects in all analyses (*15*). Speakers were positioned every 0.4 ha and playbacks were begun on 15 March, several weeks before the first eggs of the season were laid (10 April ± 1.4 days, mean ± SE). In addition to the number of offspring produced per year, we also quantified the

Fig. 2. Relative to non-predator playbacks, playbacks of predator calls and sounds broadcast throughout the 130-day breeding season reduced the numbers of eggs, hatchlings, and fledglings in successive nests of female song sparrows. All females produced two broods; and several (2 predator, 3 non-predator) attempted a third (not shown). Asterisks indicate significant differences (see table S3). Values are means ± SE.



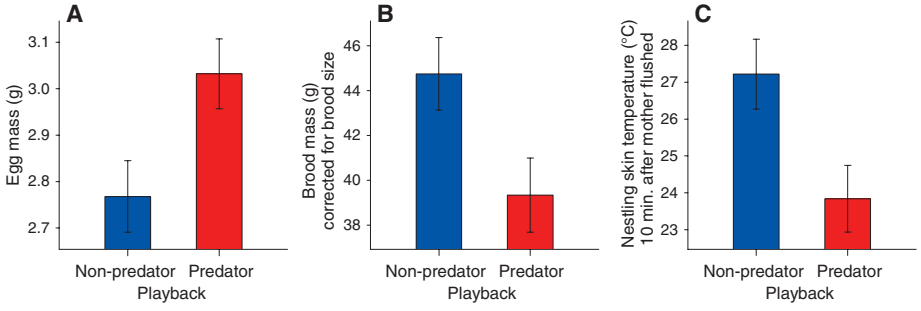


Fig. 3. Effect of perceived predation risk on offspring condition among females exposed to predator or non-predator playbacks. (**A**) Egg mass ($F_{1,22}$ = 5.99, P = 0.023). (**B**) Brood mass corrected for brood size ($F_{1,22}$ = 5.12, P = 0.034). (**C**) Nestling skin temperature 10 min after mother was flushed from nest ($F_{1,22}$ = 6.48, P = 0.018). Values are means ± SE.

effect of perceived predation risk on egg and brood mass (*4*, *7*, *8*, *15*, *16*), nestling susceptibility to thermoregulatory stress [skin temperature 10 min after mother flushed from nest (*4*, *6*, *15*, *17*)], and four measures of behavior reflective of effects on habitat use [nest site selection (*4*, *6*, *15*)], vigilance [flight initiation distance, i.e., distance of experimenter from nest when mother flushed from nest (*15*, *18*)], nest attendance [incubation bout duration (*7*, *8*, *15*, *19*, *20*)], and foraging [parental feeding visits per hour during brood-rearing (*4*, *7*, *8*, *15*, *21*, *22*)].

Females exposed to predator playbacks throughout the breeding season produced 40% fewer offspring (Fig. 1A) because they laid fewer eggs (Fig. 1B), a greater proportion of which failed to hatch (Fig. 1C), and a greater proportion of their nestlings expired (Fig. 1D). Effects were consistent throughout the season (Fig. 2). Although the effect on the number of eggs laid was more prominent in the first clutch and the effect on the proportion of nestlings expiring was more salient in the second brood, there were no significant treatment × nest interactions (Fig. 2 and table S3). Whereas predator-playback females laid fewer but heavier eggs (Fig. 3A), their broods weighed less (Fig. 3B) and were more susceptible to thermoregulatory stress (Fig. 3C).

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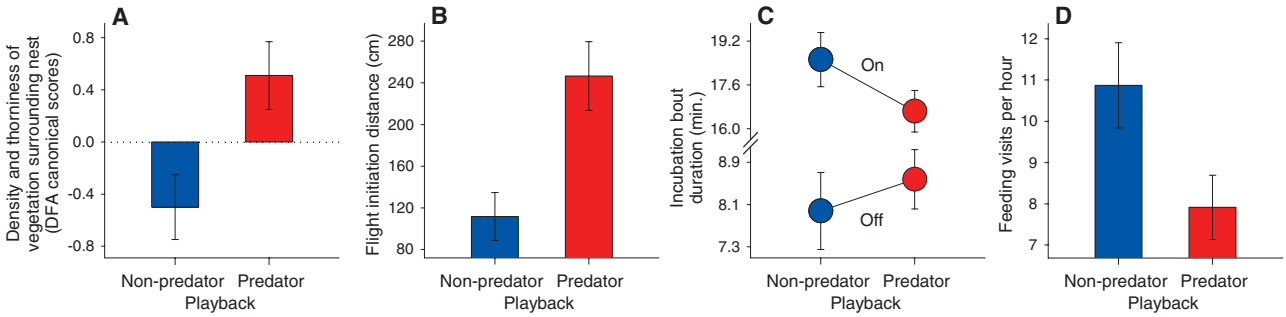

Fig. 4. Effect of perceived predation risk on parental behavior among females exposed to predator or non-predator playbacks. (**A**) Density and thorniness of vegetation surrounding nest (discriminant function analysis eigenvalue = 0.27, Wilks' $\lambda = 0.79$, $P = 0.010$). (**B**) Flight initiation distance ($F_{1,22} = 9.57$, $P = 0.005$). (**C**) Duration of incubation on- and off-bouts (repeated-measures interaction, $F_{1,22} = 4.83$, $P = 0.039$). (**D**) Parental feeding visits per hour during brood-rearing ($F_{1,22} = 5.49$, $P = 0.029$). Values are means ± SE.

Behaviorally, predator-playback females built their nests in denser, thornier vegetation (Fig. 4A), were more skittish (as measured by flight initiation distance; Fig. 4B), and spent shorter times on and longer times off the nest during incubation (Fig. 4C), and predator-playback parents made fewer feeding visits per hour during brood-rearing (Fig. 4D). Effects on all four behaviors were associated with effects on offspring number and condition (table S4) (*15*).

The effect of perceived predation risk on the population growth rate was probably even greater than that demonstrated here. In addition to the 40% reduction in the number of offspring produced, there were likely also adverse effects on juvenile and adult survival. Food restriction during brood-rearing as evidently experienced by predator-playback nestlings (Figs. 3B and 4D) can be expected to adversely affect juvenile physiology (*23*) and brain development (*24*) and so negatively affect juvenile survival. Parental stress levels were also likely elevated (*10*, *11*), with potentially negative effects on adult survival.

Our results demonstrate that the total impact of predators on song sparrow demography is greater than that due to direct killing alone (*1*–*3*). The demographic measures affected (Fig. 1, B to D) are known to all significantly affect the number of offspring produced per year when direct predation is present (*25*). Moreover, the magnitude of the effects observed (Fig. 1, B to D) corresponds to the magnitude seen in response to natural variation in predation risk, and the results of a multiannual food supplementation experiment on song sparrows (*25*, *26*) indicate that the effects observed can be expected to be robust to variation in food supply.

Only two experiments (*6*, *7*), both on songbirds and both focused on the number of eggs laid in the first clutch of the season (cf. Fig. 2), have tested the effect of perceived predation risk on the annual number of propagules produced in free-living wildlife (*1*–*4*, *8*). One experiment documented an effect on clutch size (*6*), as we did (Fig. 2), whereas the other did not, but instead found an effect on egg mass (*7*). We suggest that these all may be variants of the same response. Our results showing effects on both egg number and mass (Figs. 1B and 3A) accord with data from diverse taxa indicating that perceived predation risk may affect propagule number, mass, or both (*27*). Producing fewer, larger propagules when conditions are more challenging, as predator-playback females did (Figs. 1B and 3A), has correspondingly been observed in diverse taxa (*27*), including birds (*28*, *29*). The effects on the second nest in our experiment (Fig. 2 and table S3) illustrate that significant cumulative effects may result from modest changes in a series of demographic components. Consequently, we propose that further progress can be made by focusing more on cumulative effects rather than specific components (*1*–*3*).

Our demographic results demonstrate that the effects of perceived predation risk on the behavior and physiology of prey can scale-up to affect survival in free-living wildlife (Fig. 1, C and D) in addition to affecting the number of propagules produced (Fig. 1B) (*6*). The pathway from the prey's perception of predation risk through changes in behavior and physiology to effects on demography no doubt involves more behaviors than we measured in addition to various physiological responses (*1*–*3*). Most links in this pathway likely also have multiple facets (*2*, *11*). For example, both nest site selection and parental provisioning evidently affected nestling mortality (table S4) at different times during brood-rearing. The proportion of nestlings that expired (Fig. 1D) was more closely associated with thermoregulatory stress (Fig. 3C) earlier in brood-rearing, whereas it was more closely associated with brood mass (Fig. 3B) later (table S5). This suggests that the effect of perceived predation risk on nest site selection (Fig. 4A), and thus presumably microclimate (*4*, *6*, *15*), affected nestling mortality while the nestlings were still naked and unable to conserve heat, whereas the effect of perceived predation risk on parental provisioning (Fig. 4D) was more important later in brood-rearing.

We expect that demographic effects comparable to those shown here will be found in many other systems once more such demographic experiments are conducted, because the behavioral responses we observed concerning nest site selection (Fig. 4A), skittishness (Fig. 4B), and feeding visits (Fig. 4D) correspond to those reported in behavioral studies on diverse species (*1*, *4*, *6*–*9*, *15*, *18*, *21*, *22*). The type of predator may be expected to be an important factor in shaping these responses (*4*, *21*). Our manipulation included predators of both adults and offspring (table S1). The sole comparable experiment (*4*, *8*) evaluating effects on incubation behavior manipulated only predators of offspring (*7*). The response we observed (Fig. 4C) accorded with nest attendance being influenced by the parent's perception of the risk to itself while it is off the nest foraging (*20*). Consistent with there being a difference in the type of predator manipulated (offspring only), the response in the previous study differed (*7*). Whether such varying responses to different types of predators have different effects on demography merits further exploration (*4*, *21*).

Our experimental results demonstrating that the prey's perception of predation risk alone is powerful enough to affect the population growth rate of free-living wildlife corroborate the results of recent correlative studies on elk and wolves (*5*, *30*), snowshoe hares (*3*), and dugongs and sharks (*5*), and complement the results of the aforementioned demographic experiments on songbirds (*6*, *7*). Consequently, the total impact of predators on wildlife populations may be significantly underestimated if the costs of perceived predation risk are not duly considered (*1*–*3*).

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Supporting Online Material

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Arabidopsis EDS1 Connects Pathogen Effector Recognition to Cell Compartment–Specific Immune Responses

Katharina Heidrich,[1] Lennart Wirthmueller,[1]* Céline Tasset,[2] Cécile Pouzet,[3] Laurent Deslandes,[2] Jane E. Parker[1]†

Pathogen effectors are intercepted by plant intracellular nucleotide binding–leucine-rich repeat (NB-LRR) receptors. However, processes linking receptor activation to downstream defenses remain obscure. Nucleo-cytoplasmic basal resistance regulator EDS1 (ENHANCED DISEASE SUSCEPTIBILITY1) is indispensible for immunity mediated by TIR (Toll–interleukin-1 receptor)–NB-LRR receptors. We show that *Arabidopsis* EDS1 molecularly connects TIR-NB-LRR disease resistance protein RPS4 recognition of bacterial effector AvrRps4 to defense pathways. RPS4-EDS1 and AvrRps4-EDS1 complexes are detected inside nuclei of living tobacco cells after transient coexpression and in *Arabidopsis* soluble leaf extracts after resistance activation. Forced AvrRps4 localization to the host cytoplasm or nucleus reveals cell compartment–specific RPS4-EDS1 defense branches. Although nuclear processes restrict bacterial growth, programmed cell death and transcriptional resistance reinforcement require nucleo-cytoplasmic coordination. Thus, EDS1 behaves as an effector target and activated TIR-NB-LRR signal transducer for defenses across cell compartments.

Plant nucleotide binding–leucine-rich repeat (NB-LRR) proteins constitute a large family of intracellular receptors mediating strain-specific disease resistance (*1*). Recognition of pathogen effectors causes NB-LRR activation through adenosine triphosphate (ATP)–driven conformational changes that lead to induction of antimicrobial defenses and localized host programmed cell death (*1*, *2*). Structural counterparts of NB-LRRs (called NACHT- or NOD-LRRs) regulate innate immune responses and apoptosis in mammalian cells (*3*), but in neither system are the mechanisms connecting receptor activation to defense reprogramming well understood. A major class of plant NB-LRR receptor with N-terminal TIR (Toll–interleukin-1–receptor domain) homology has evolved to intercept effectors from many different pathogen types (*1*). The nucleo-cytoplasmic lipaselike protein ENHANCED DISEASE SUSCEPTIBILITY1 (EDS1) controls basal immunity by restricting growth of virulent pathogens (*4*–*6*). EDS1 also signals downstream of activated TIR-NB-LRR receptors, to control host cell death and transcriptional mobilization of defense pathways (*7*–*9*). In *Arabidopsis*, TIR-NB-LRR receptor RPS4 recognizes a *Pseudomonas syringae* type III secreted effector AvrRps4 (*10*), and EDS1 nuclear accumulation is a prerequisite step for RPS4 resistance and associated transcriptional reprogramming (*11*). Coordination of the nucleo-cytoplasmic EDS1 pools through nuclear pore complexes is necessary for full TIR-NB-LRR immunity, which implies a need for EDS1 mobility inside cells (*6*, *11*, *12*).

We examined the molecular and subcellular relation between bacterial AvrRps4 protein and *Arabidopsis* RPS4 and EDS1. After delivery to plant cells, AvrRps4 is cleaved to release an 11-kD C-terminal fragment ($AvrRps4^C$), which is necessary and sufficient for eliciting RPS4 immunity (*13*, *14*). Although RPS4 associates mainly with endomembranes (*9*), RPS4 nuclear accumulation and genetic cooperativity with a nuclear WRKY transcription factor domain–containing TIR-NB-LRR receptor, RRS1, is required for AvrRps4-triggered immunity (*9*, *15*–*17*). Cytoplasmic membranes have emerged as an important cell compartment in which RPS4 and at least one other TIR-NB-LRR protein (SNC1) are constrained by the tetratricopeptide repeat (TPR) protein SRFR1 (SUPPRESSOR OF rps4-RLD1) and a SRFR1-interacting cochaperone SGT1 to prevent autoimmunity (*18*, *19*).

We determined in which subcellular compartment $AvrRps4^C$ activates host defenses by expressing AvrRps4 with a C-terminal yellow fluorescent protein (YFP) fusion alone or YFP attached to a eukaryotic nuclear localization (NLS), a nuclear export (NES), or respective mutated nls and nes sequences (*20*). In *Agrobacterium*-mediated transient expression assays of *Nicotiana benthamiana* leaves, $AvrRps4^C$-YFP (as well as $AvrRps4^C$-YFP-nls and $AvrRps4^C$-YFP-nes) displayed a nucleo-cytoplasmic distribution, monitored by live-cell imaging (fig. S1). $AvrRps4^C$-YFP-NLS was detected only in nuclei and NES-tagged $AvrRps4^C$-YFP in the cytoplasm of *N. benthamiana* cells, which suggested that $AvrRps4^C$ can be forced into either compartment (fig. S1). Expression of the same constructs in *Arabidopsis* stable transgenic plants of a non-responding *eds1* mutant in *Arabidopsis* accession Columbia (Col *eds1-2*) conferred similar $AvrRps4^C$ distribution patterns (Fig. 1A), although YFP fluorescence was considerably lower than in the transient assays. None of the *eds1* transgenic lines displayed stunting or necrosis, which are hallmarks of autoimmunity (*7*, *9*, *21*), consistent with *eds1* failing to respond to $AvrRps4^C$ (Fig. 1B). Multiple independent transgenic *eds1* lines for each construct were crossed with wild-type Col to introduce functional *EDS1*. All F_1 progeny produced severely dwarf plants that died

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