Quantifying the success of prey crypsis, aposematism and evasiveness in avoiding avian predators' attack

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Open research statement

The data that support the findings of this study are available from the corresponding author upon reasonable request and will be archived on Zenodo after acceptance.

Keywords

Learnt avoidance behaviour, predator-prey-interaction, anti-predator defences, predation sequence

Abstract

1. Antipredator defences typically act at specific stages along the predation sequence, i.e., during prey encounter, identification, approach, and subjugation. However, the effectiveness of such defences has seldom been contrasted and quantified in parallel using an experimental setting with wild predators.
2. Within aviaries installed in two localities in northeastern Peru, we recorded the responses of avian predators to three antipredator defences, crypsis, aposematism and evasiveness, each expressed in three butterfly species. We examined both immature and adult birds from forest and urban environments, including species having diets ranging from fully insectivorous to omnivorous.
3. We confirmed the expectations that cryptic butterflies (Nymphalidae: Satyrinae: Euptychiina) largely remained undetected, whereas aposematic *Heliconius* (Nymphalidae: Heliconiinae) were frequently noticed but seldom attacked. Evasive *Spicauda* (Hesperiidae: Eudaminae) were frequently detected and attacked but exhibited higher evasion rates likely owing to their fast flight.

4. Bird life stage dramatically influenced defence effectiveness. Immature birds were more prone to attacking *Heliconius*, possibly due to being in early learning stages of local aposematic cues. We also found evidence that predation pattern differs between bird families, making more adept predators more likely to attack defended prey (i.e., Vireonidae).
5. Our findings illuminate the nuanced dynamics of predator-prey interactions in complex tropical environments, highlighting the intricate behavioural responses of wild predators and how they shape the co-existence of diverse antipredator defences.

Introduction

Butterflies are an abundant prey that rely on a large array of antipredator defences acting at different stages in the predation sequence. For example, cryptic butterflies avoid being attacked by reducing the probability of predator encounter and detection by background matching (Feltmate & Williams, 1989; Vallin et al., 2006; Pinheiro & Campos, 2019; Pembury Smith & Ruxton, 2021). In the wild, this can be evaluated using wing damage, although the actual predation risk of cryptic prey cannot be thoroughly assessed (Molleman et al., 2020). Acting later in the predation sequence, unpalatable butterflies usually advertise their unprofitability by displaying aposematic cues that predators might learn to avoid (e.g., Heliconiini and Ithomiini butterflies; Arias et al., 2016; Chouteau et al., 2016; Willmott et al., 2017; Chouteau et al., 2019). However, in experiments designed to measure the attack rates towards aposematic prey, the possibility of rejection after an initial attack is difficult to assess (Mappes et al., 2014; Seymoure et al., 2018). Finally, evasive flight behaviour (Pinheiro & Freitas, 2014) and deflective structures may prevent deadly attacks after being spotted. The effectiveness of such a behaviour

has been difficult to evaluate, but recent efforts shed light on predators preferring to attack non-evasive dummy models (Páez et al., 2021; Linke et al., 2022).

No antipredator defence offers complete protection against predators, especially in ecosystems harbouring a large diversity of predator species. However, our understanding on how wild predators respond to simultaneously encountered defence behaviours is limited by the scarce number of experimental studies aiming to quantify naturally occurring predation in controlled environments (Pinheiro & Campos, 2019; wild predators but dummy prey - Guerra et al., 2024; Dell'aglio et al., 2016). By carrying out behavioural experiments in large aviaries with wild birds that include insects in their diets, we aim to understand how predators decide to engage in attacking behaviour when preys with distinct antipredator defences are presented simultaneously, as well as how successful different prey species (with different anti-predator defences) are when avoiding avian predators. The prey that we study are butterflies with antipredator defences believed to act at different stages of the predation sequence: 1) Euptychiina (Nymphalidae: Satyrinae: Satyrini), which likely relies on crypsis to avoid being detected, 2) *Heliconius* (Nymphalidae: Heliconiinae: Heliconiini), which likely relies on aposematism and Müllerian mimicry to avoid being identified as profitable prey, and 3) *Spicauda* (Hesperiidae: Eudaminae: Eudamini), which likely relies on evasive behaviour to avoid being caught. We investigate variations in predator responses, such as those due to predator life stage (immature vs. adult), bird diets and bird taxonomic family.

We postulate the following main predictions stemming from predator-prey-interaction theory:

(I) **Probability of remaining undetected:** cryptic butterflies (i.e., the dull brown colouration of Euptychiina and *Spicauda*) have lower probabilities to be detected by the bird compared to a conspicuous butterfly (i.e., the aposematic *Heliconius*).

(II) **Probability of being rejected upon identification:** butterflies that advertise unprofitability by the means of conspicuous colour patterns (i.e., *Heliconius*) have a higher probability of being sight rejected, without active attacking behaviour, by a predator that has learned the visual cues associated to unprofitable prey (as opposed to, e.g., the presumably slow flying, dull coloured and palatable Euptychiina).

(III) **Probability of capture avoidance upon predator attack:** Once a bird has engaged in prey pursuing, butterflies with evasive flights are harder to catch compared to slow-flying butterflies (e.g., Euptychiina or *Heliconius*).

(IV) **Probability to survive upon predator attack:** Once a bird has engaged in prey pursuing, butterflies with structural/chemical defences (such as the sturdy wings of unpalatable *Heliconius* or the hindwing tails of *Spicauda*) survive attacks by being released with non-fatal damage.

Methods

Study location

The experiments were carried out during the rainy season of 2021/22 (October – February) and the dry season of 2022 (June – September). The two study locations were 1) within the city limits of Tarapoto, Peru (6.47°S, 76.35W), and 2) within a privately protected area, the Urahuasha sector (6°27′S 76°20′W), near the external border of the "Área de Conservación Regional Cordillera Escalera", San Martin, Peru. The first study site was characterised by significant

human intervention, and the second site was characterised by secondary growth forest (~50 years old) with very limited human influence.

Birds

In both study sites, we installed dull-coloured mist nets near roads and pathways within scrubs, open secondary forest, forest edges or hill crests. All trapping locations are listed in Supplement 1. Mist nets were opened during early morning hours (~7AM) and checked in hourly intervals until dusk (~5PM). The caught birds were identified to the species level, the age was determined as immature or adult, and all individuals were photographed. Immature birds, which are independent but sexually immature, were determined by inspection of non-definitive plumage and coloured commissures. Selected bird species for the behavioural experiments were documented to feed on butterflies (Pinheiro & Cintra, 2017), were classified as insectivorous in the literature (Egg et al., 2010), or their diet included a high proportion of insects based on local expert knowledge. The birds' diet was classed as either fully insectivorous or other (i.e., omnivorous, invertebrates, frugivorous/insectivorous). All other birds were marked and released. The experimental birds were kept in captivity for a maximum of 30 hours at the urban site and for a maximum of 12 hours in the forest site. Every bird in the cages had free access to water and fresh local papaya.

Butterflies

We used three phenotypes that represented typical antipredator defences in butterflies (Figure 1). **(I)** As representatives of cryptic prey, we studied medium-sized Euptychiina, consisting of mainly *Cissia penelope* and related, syntopic species in the genera *Cissia*, *Magneuptychia* and *Yphthimoides.* **(II)** As representatives of unpalatable, aposematic prey, we studied *Heliconius*

erato, and if not possible to find any in a given day, we used the co-mimetic *H. melpomene*. **(III)** As representatives of prey relying on evasive flight behaviour to avoid predation, we studied *Spicauda simplicius*, and if not possible to find any in a given day, we used the morphologically similar and syntopic *S. teleus*, *S. tanna*, and *S. procne*. Importantly, all taxa classified in each of the three study phenotypes had highly similar morphologies, behaviours and co-occurred in the same microhabitats.

Butterflies were caught using entomological nets around the study sites. Their species identities were determined to the species level, with Euptychiines sometimes only to the genus level. The live butterflies were transported to the field station in glassine envelopes. One individual of each phenotype group was then released into mesh cages (50×50×50 cm) containing fake plastic flowers filled with sugar water. Each butterfly individual was used for a maximum of three predation experiments, or for a maximum of 48 hours to avoid noticeable fatigue. Afterwards, butterflies were sacrificed and stored in silica gel for further morphological identification.

Behavioural experiments in aviaries

At each study location and in open space to obtain similar luminosity, we constructed an experimental aviary (H×W×L = 4×2×4 m) made of green garden mesh and plumbing tubes (see Supplement 2 for photos). We installed bamboo perches for the birds to rest on at ~50 cm below the aviary ceiling. We installed two video cameras (GoPro HERO6, San Mateo, USA; set to 1080p, 60 fps and the wide lens setting) on the two front sides of the aviary at ~1 m height, facing each other, allowing for the complete capture of the inside of the aviary. The experiments were conducted from ~7 AM to ~6 PM depending on availability of birds and butterflies.

The behavioural experiments consisted of simultaneously presenting the three butterfly phenotypes to a bird inside the aviary and recording the behavioural responses of predator and preys. After starving for 60 minutes, a single bird was released into the aviary, where it was left alone for 30 minutes to destress and adjust to the aviary surroundings. Afterwards, through a small opening in the door, the three butterflies were released into the aviary. The cameras recorded the experiments for 60 minutes, during which there were no people around the aviary to avoid any additional stress for the bird. At the end of the experiment, we caught any remaining live butterflies and surveyed the ground for any detached wings. After cutting two tail feathers for identification, the bird was released before dark in favourable weather conditions. Any recaught birds were not used for a second experiment. The predation experiment was counted as invalid if major long-term disturbances were present, including sudden rainfall, the appearance of cats or monkeys in the surroundings, the bird or any of the butterflies escaped during the experiment, or the bird showed signs of fatigue and stress, i.e., no movement, unable to leave the ground, or having a constant and erratic flights from side to side.

Video processing

We aligned the two cameras in time to allow for the simultaneous observation of both cameras, using DaVinci Resolve 18 (Blackmagic Design, Melbourne, Australia). We noted the behavioural responses of birds and butterflies as timestamps (Figure 2), recording the start and end of stages in the predation sequence: encounter, identification, approach, subjugation and consumption. Each bird-butterfly combination was assigned the highest observed stage within the predator-prey-interaction. Birds which did not interact with any butterfly were excluded from analysis (i.e., no sight rejection or attack). We also recorded the approximate time the bird and

each butterfly co-occurred at two heights in the aviary: ground level, 0–200 cm, and ceiling level, 200–400 cm.

Chance of predator-prey encounter

To estimate the encounter probability, we calculated the co-occurrence rate of the bird and each butterfly, using:

$$(1)\ Co = Bird_{low} \times Butterfly_{low} + Bird_{high} \times Butterfly_{high}$$

Where, the first term is the proportion of experimental time the bird and a butterfly co-occurred in the ground level (0– 200 cm) and the second term is the proportion of time they co-occurred in the ceiling level (200–400 cm). The *Co* index, thus, ranges between 0 and 1, where 0 is a complete spatial mismatch between the bird and a butterfly (very unlikely the predator encounters the prey) and 1 is a complete overlap (highly likely that the predator encounters the prey).

Predictors of bird behaviours to prey phenotypes: logistic regressions

To identify major patterns in bird behaviour (sight rejection or attacking a prey) in relation to prey type, bird age (immature or adult), and diet (fully insectivorous or mixed diet), we used generalized linear mixed models (GLMMs) with binomial distribution, as implemented by the *´glmer´* function from the *lme4* R package (Bates et al., 2015). Bird individual was included as a random effect to account for individual variability. To compare the sight rejection and attacking behaviours using logistic regressions, we transformed each bird-butterfly interaction into a binomial outcome based on the highest observed behavioural stage (Figure 2). Two separate models were used:

$$\text{(2a)}\ logit(P(Y_{sight-reject} = 1)) = \beta_0 + \beta_{butterfly_type} \times X_{butterfly\ type} + \beta_{age} \times X_{age} + \beta_{diet} \times X_{diet} + u_{birdID}$$

$$\text{(2b)}\ logit(P(Y_{attack} = 1)) = \beta_0 + \beta_{butterfly_type} \times X_{butterfly\ type} + \beta_{age} \times X_{age} + \beta_{diet} \times X_{diet} + u_{birdID}$$

Where Y is the probability of a butterfly to be sight rejected or attacked by a bird; β_0 is the general tendency to sight reject or attack; $\beta_{butterfly_type} \times X_{butterfly\ type}$, $\beta_{age} \times X_{age}$ and $\beta_{diet} \times X_{diet}$ represent the effect of butterfly type, age and diet on this probability, respectively, and u_{birdID} accounts for variation among individual birds. For the sight-rejection model (2a), the response variable was coded as 1 for sight rejection ($N_{det} – N_{att}$, i.e., by being ~10 cm close to the butterfly without attacking behaviour) and 0 for attacking behaviour (N_{att}). In the attacking model (2b), the response variable was coded as 1 for active attacking (N_{att}), and 0 for all other responses ($N_{det} – N_{att}$ and $N – N_{det}$, i.e., sigh rejection or no interaction at all). Additional models incorporating variables such as season, locality, and bird family were also tested and are described in Supplement 3.

Probabilities of attack deterrence at different stages in the predation sequence

We used log-likelihood tests to compare the probabilities of a prey to (1) remain undetected, (2) get sight-rejected upon detection, (3.1) get attacked, (3.2) avoid capture during a predator attack and (4) survive a predator attack. The tests were performed using the birds that detected at least one butterfly prey (any interaction not: $N – N_{det}$), i.e., birds that were ~10 cm close to the prey and birds that showed active attacking behaviour (Figure 2). Further tests were also performed using the data subset by predator age (immature or adult) (additional models for season, wet or dry, and for habitat, urban or forest, were also conducted, see Supplement 4). We calculated the

log-likelihood of models depicting the number of events within each predation sequence stage for a particular prey phenotype compared to others, following the method implemented in Mérot et al. (2015), Willmott et al. (2017) and Páez et al. (2021):

$$(3)\ log_{10}(L) = \sum_i [a_i log_{10}(P_i) + (N - a_i) log_{10}(1 - P_i)] + K$$

Where *i* represents the prey type (Euptychiina, *Heliconius* or *Spicauda*); *N* denotes the total count the prey was presented, a_i is the count of interactions with the prey type *i*; P_i is the attack probability for each prey type *i*; and *K* is a constant that cancels out when comparing models. For each stage of the predation sequence, we compared five competing models: all prey having different probabilities of interaction ("all different"), the same probabilities ("all equal"), and two of the prey phenotypes having the same probability but different from the third phenotype ("Euptychiina different", "*Heliconius* different" and "*Spicauda* different"). The model with the lowest AICc (corrected Akaike information criterion) value was selected as the best model, while any model within 2 units of AICc was not rejected. We calculated Akaike weights following Burnham & Anderson (2013) to illustrate the differences among the five tested models in each stage of the predation sequence.

Description of the likelihood-based model tests for each stage in the predation sequence

(1) Probability to remain undetected: We compared the sum of occasions for each prey phenotype where the bird and the butterfly did not interact (i.e., the prey was not encountered nor detected) throughout the whole experiment ($N - N_{det}$) against the total number of tested birds (N).

In addition to the likelihood-based comparison, we compared *Co* index values between butterflies using a pairwise Wilcoxon signed-rank tests with Bonferroni correction (for multiple

comparisons) as normal distribution of the data was rejected using the Shapiro-Wilk test ($p < 0.0001$).

(2) Probability of being rejected upon detection: We compared the sum of events for each prey phenotype where the bird was close to the butterfly (~10 cm) but did not engage in any obvious attacking behaviour ($N_{det} – N_{att}$), in comparison to all birds that did enter the predation sequence for at least one butterfly (N). We assumed that the birds in spatial proximity to a butterfly were able to spot the prey but decided not to pursue any attacking behaviour (sight rejection).

(3) Targeting probability and capture avoidance: This involves two steps, **(3.1)** the chance of getting attacked in the first place and **(3.2)** the ability to avoid being caught when attacked. First, **(3.1)** we compared the sum of events where the butterfly was actively attacked by the bird depending on the butterfly type, regardless of the outcome (N_{att}), in comparison to all birds that entered the predation sequence with this prey phenotype (N_{det}). Second, **(3.2),** to determine whether prey phenotypes differ in their ability to avoid being captured, we additionally computed the sum of events where the butterfly was not captured ($N_{att} – N_{sub}$) in comparison to the sum of birds showing active attacking behaviour to each prey phenotype (N_{att}).

Lastly, we determined for each bird and butterfly combination the time for the first instance of attacking behaviour (no attack, equals 60 minutes) to compare any preference in engaging to pursue different butterfly types. As a normal distribution of the data was rejected using the Shapiro-Wilk test ($p < 0.0001$), we performed pairwise Wilcoxon signed-rank tests with Bonferroni correction (for multiple comparisons) to compare initial attack time distributions between butterflies.

(4) Probability to survive upon predator attack: We compared the sum of failing to kill (N_{att} – N_{cons}) for each prey phenotype in comparison to the number of actively attacked butterflies per phenotype (N_{att}).

Statistics (except log-likelihood tests, which were implemented in a spreadsheet; Supplement 4) were done using R v. 4.3.0 (R Core Team, 2023). Graphics were generated using the R package *ggplot2* v. 3.4.3 (Wickham, 2016).

Results

We performed 262 experiments, of which 216 were deemed valid. There were 65 valid experiments in the urban environment and 151 experiments in the forested habitat, of which 70 were performed in the wet season and 81 in the dry season. Due to time and logistics constraints, we were not able to carry out experiments in the urban environment during the dry season.

In total, we studied birds classified in 41 species from 13 families. The number of bird species that include insects in their diet was higher in the forested habitat than in the urban environment (urban/wet season = 12 species; forest/wet season = 19 species; forest/dry season = 23 species) (Supplement 5). Complete lists of birds observed at both localities, including photographs of caught birds, are accessible through Field Museum field guides (Navarro et al., 2023, Hernández Mejía et al. *in review*). In the urban environment, 68% of all birds were classed as adults and only 32% were immatures having a maximum age of 2 years. In the forested habitat, during the wet season only 29% of all birds were adults and 71% were immatures, whereas during the dry season, only 16% of birds were adult and 84% were immatures (for species-level ratio see Supplement 5).

General differences between prey types and bird families

When only considering birds that sight rejected or attacked at least one butterfly, nearly 70% of them did not interact with Euptychiines ($N - N_{det}$), whereas this proportion was notably lower for *Heliconius* (34%) and *Spicauda* (18%). Sight rejection ($N_{det} - N_{att}$) was most prevalent toward *Heliconius*, with almost 50% of birds exhibiting this response, followed by *Spicauda* (18%) and Euptychiines (9%). *Spicauda* was most frequently attacked by birds (N_{att}, 40%), while attack rates for Euptychiines and *Heliconius* were comparable at 21% and 18%, respectively. Only 6% of all birds caught (i.e., N_{sub}, killing or releasing it afterwards) *Heliconius*, compared to 16% for *Spicauda* and 12% for Euptychiines (Supplement 6). Overall mortality rates (N_{cons}) were relatively similar across all three prey phenotypes, with Euptychiines at 7%, *Heliconius* at 4%, and *Spicauda* at 9%.

Among bird families, Furnariidae, were most effective in targeting Euptychiines, with 21% successfully catching them, while Thraupidae were the least effective, with only 8% exhibiting some interaction (i.e., at least sight rejection). Bird families showed similar probabilities of sight rejecting *Heliconius*, ranging between 30-50%. Tyrannidae and Vireonidae, two highly insectivorous families and skilled flycatcher hunters, were the only birds that did not sight reject *Spicauda*, with Vireonidae displaying the highest success in capturing them among all bird families (Supplement 6).

Predictors of bird behaviours to prey phenotypes: logistic regressions

The GLMM for sight rejection behaviour revealed that birds more frequently rejected *Heliconius* compared to Euptychiines (Estimate = 14.71, $p < 0.001$), while there were no significant differences for *Spicauda* ($p = 0.882$), bird state ($p = 0.479$), or diet ($p = 0.745$). There was substantial individual variation (SD = 17.47). In the GLMM for attacking behaviour, birds were

significantly more likely to attack *Spicauda* compared to Euptychiines (Estimate = 1.04, $p < 0.001$). No significant differences were observed for *Heliconius* ($p = 0.408$), bird state ($p = 0.617$), or diet ($p = 0.699$). Individual variation in attacking behaviour was low (SD = 0.94). The full model outputs and additional models including bird family, habitat and season can be found in Supplement 3. Notably, Vireonidae birds were significantly less likely to sight-reject ($p = 0.028$) and more likely to attack ($p = 0.035$) prey butterflies compared to other bird families. This interaction was barely significant for Tyrannidae (sight rejection; $p = 0.095$ and attacking; $p = 0.062$).

Likelihood-based comparisons along the predator-prey interactions

Results for log-likelihood tests for the total dataset, immatures and adults can be found in Figure 4 and Table 1. Full calculation and tests for habitat (forest vs. urban) and season (dry vs. wet) can be found in Supplement 4. Graphs for season and habitat can be found in Supplement 7.

(1) Probability to remain undetected: Across all bird families in different habitats and seasons, there was a preference for foraging near the ceiling level (Figure 3(a)). Antbirds (Thamnophilidae, 23 individuals and 9 species) spent most of the time (66%) foraging near the ground, whereas Vireonidae (9 individuals and 2 species) spent on average only 14% of their time near the ground. Butterflies, on average, differed in their stratum selection (Figure 3(b)). Euptychiines tended to occur most of the time (55%) near the ground, whereas *Spicauda* and *Heliconius* spent 78% and 83% of their time, respectively, in the ceiling level, which suggest that they would have been highly detectable for most of the studied bird groups.

Our likelihood-based model test supported the “Euptychiines different” scenario (Akaike weight: 0.51). Birds were overall least likely to encounter them compared to *Heliconius* and *Spicauda*

(Figure 4, Table 1). Additionally, “all different” was the second-best model (ΔAICc = 0.18), with birds most likely to encounter *Heliconius*. For adult birds, “Euptychiines different” and “Spicauda different” had almost equal Akaike weights (0.32 and 0.31 respectively), both scenarios suggesting that birds were least likely to encounter the Euptychiines and most likely *Spicauda*. However, the “all equal” model (no difference among butterfly species) was not statistically rejected (Akaike weight: 0.22; ΔAICc = 0.69). For immature birds, “all different” was the best model, with Eptychiines the least, *Spicauda* average and *Heliconius* the most likely to be encountered by them. Additionally, “*Heliconius* different” was not fully rejected (ΔAICc = 1.95).

Our measure of encounter chance for the bird and each butterfly type (*Co* index, Figure 5(a)) suggested that Euptychiines and *Heliconius* ($p < 0.0001$; Euptychiina: $Co = 0.477 \pm 0.371$ and *Heliconius*: $Co = 0.641 \pm 0.344$) as well as Euptychiines and *Spicauda* ($p = 0.0047$; *Spicauda*: $Co = 0.600 \pm 0.351$) differed significantly in their spatial co-occurrence, whereas no difference was detected between *Spicauda* and *Heliconius* ($p = 0.283$). This confirmed that while *Heliconius* and *Spicauda* typically co-occurred in the upper part of the aviary with most birds, Euptychiines preferred to occupy the ground level, thus, reducing their probability of being encountered.

(2) Probability of being rejected upon identification: The best-selected model was "*Heliconius* different" (Akaike weight = 0.50) with "all different" being the second-best model (Akaike weight = 0.50, ΔAICc = 0.02; Figure 4, Table 1). Both models suggested that most birds sight-rejected *Heliconius*, followed by *Spicauda* and Euptychiines. A similar pattern was supported by both immatures and adults, with immatures exhibiting higher sight-rejection rates towards all

butterfly types. *Spicauda*, even though it co-occurred at high rates with most birds in the aviaries, was sight-rejected at a higher rate (the “all different” scenario) than Euptychiines.

(3.1) Targeting probability: The "*Spicauda* different" was identified as the best model (Akaike weight = 0.62, Figure 4, Table 1), suggesting that birds more frequently attacked *Spicauda* than Euptychiines and *Heliconius*. This is in line with our observations of birds often attacking *Spicauda* multiple times until they captured and subjugated it (Figure 5(b)); the ratio of the number unsuccessful attacks to the sum of all birds attacking a prey is highest for *Spicauda* with 2.05, followed by *Heliconius* with 1.61 and Euptychiines with 0.92. The model "all different" was not rejected (Akaike weight = 0.25, ΔAICc = 1.77), suggesting that Euptychiines were attacked more frequently than *Heliconius* by birds that detected a butterfly prey. For adult birds, only "all different" and "*Spicauda* different" models were not rejected, indicating *Spicauda* as the primary target followed by Euptychiines. Conversely, immatures showed a more evenly distributed attack rates, with "all equal" emerging as the best model, whereas the model "all different" was rejected (ΔAICc = 2.24), highlighting a less selective predator attack patterns for immatures.

(3.2) Capture avoidance: “All equal" was the best model, suggesting that all butterflies showed similar likelihood of evading capture upon predator attack (Akaike weight = 0.37, Figure 4, Table 1). For immatures, additional models ("*Heliconius* different," and "Euptychiines different") were selected with a ΔAICc between 1 and 2, indicating varying capture efficiency. Conversely, for adult birds, the only selected models were "all equal” and “Euptychiina different”, suggesting similar capture rates for both *Heliconius* and *Spicauda*, with Euptychiines being the easiest to catch

To supplement the likelihood-based tests, we compared the times of first predator attack among butterfly types. The Wilcoxon test suggested that the times to attack differed between *Spicauda* and *Heliconius* (*Spicauda* 32 ± 22.4 min; *Heliconius* 46.6 ± 22.3 min; $p < 0.001$) as well as between *Spicauda* and Euptychiines (Euptychiina 47.9 ± 19.6 min; $p < 0.001$) (Figure 5(c)), confirming that *Spicauda* was attacked significantly earlier compared to the other two prey types.

(4) Probability to survive upon predator attack: The "all equal" model was preferred (all other models with a ΔAICc > 1.67). For immatures, the models "Euptychiines different" and "*Spicauda* different" were not rejected, suggesting that Euptychiines were more likely to be subjugated once a predator attacked it compared to the evasive *Spicauda* or the aposematic *Heliconius*. This pattern was absent for adults, with the "all equal" scenario being the only model with ΔAICc < 2.

Discussion

Our study tested four predictions of the predation sequence theory related to the fitness of antipredator defences during the prey encounter, identification, attack and subjugation stages. We studied tropical birds that include insects in their diets and live butterflies displaying antipredator defences (crypsis, aposematism and evasiveness) thought to best function at different stages of the predation sequence (Kikuchi et al., 2023). By using a controlled setting in aviaries, we found that the overall survival rates among the types of prey across all stages of the predation sequence were relatively equal. Overall, we were able to detect strong signals for the efficiency of 1) crypsis in minimizing the chances of encountering and detecting prey, 2) aposematism driven by Müllerian mimicry in deterring attack during the identification stage, and

3) fast and evasive flight behaviour in reducing the probability of subjugation once a predator engaged in attack.

We provide strong evidence from wild insectivorous birds and co-occurring butterfly prey that the life stage of predators, more than their diets, strongly influence the fitness of antipredator defences. Aposematism did not significantly deter attacks by immature birds whereas the evasive ability of *Spicauda* was more successful in avoiding attack by immatures than by adult birds. Although we corroborated several theoretical expectations stemming from the predation sequence, our work also provides strong evidence that the higher number of insectivorous bird species and larger proportion of immatures to adults in the mature secondary tropical forest compared to the urban environment, were the main drivers for the overall even mortality rates among the three butterfly types.

Defence efficiency along the predation sequence

Probability to remain undetected: Cryptic species often remain motionless for prolonged periods of time, are hard to spot (Stevens & Merilaita, 2009; Seymoure et al., 2018; Ruxton et al., 2018) and forage close to the ground (Burd, 1994). Euptychiines are characterized by dull wing colourations and often flying near the ground, which, indeed, resulted in them being the least likely prey phenotype to be encountered by birds in our study. Conversely, while *Spicauda* also has a uniform brown appearance and might be considered cryptic, their conspicuous flight behaviour often renders them in spatial proximity to most avian predators, thus, possibly indicating a relaxed selection for crypsis and a reliance on their evasive flight abilities or hindwing tails to evade predation upon attack (Linke et al., 2024).

By using 13 bird families that are either fully or partly insectivorous, we detected a strong preference of birds towards the upper layer of the aviary (200 - 400 cm). We noticed that the detection rates of prey varied in experiments carried out in the forest and urban environments. However, this might be due to differences in species specific height preferences, bird species having different height preferences depending on the habitat, or the different abundances of immatures relative to adults in each habitat. As there was little overlap in both species and age distribution between habitats in our study, we are unable to determine why prey detection rates seem to have shifted depending on the habitat (Supplement 4). Future studies that survey birds for longer time and in different localities are needed to incorporate species that occur in various habitats (e.g., urban and forest) as well as to include enough adults and immatures per species to allow for comparisons in foraging strategies on a species level. Additionally, we were not able to catch other birds with skilled hunting capabilities of flying insects, such as Galbulidae (Jacamars), and future experiments may benefit from incorporating them, as done in other observation studies (Chai, 1986; Langham, 2006; Pinheiro & Campos, 2019).

Altogether, our results emphasize that immature birds, possibly due to their naivety, are less prone to detect cryptic prey compared to more experienced adults (Mappes et al. 2014). Overall, the effectiveness of crypsis in evading detection is evident, particularly in diverse environments where immature birds dominate, as in our study location in mature secondary forest habitat.

Probability of being rejected upon identification: Our results show that regardless of bird diet and species, predators actively sight rejected *Heliconius* (i.e., assuming that predator and prey being closer than ~10cm and there was no attack behaviour), suggesting that the wild birds recognised the aposematic local patterns of unprofitable prey (e.g., Finkbeiner et al., 2014; Dell'aglio et al., 2016). By contrast, immature birds, presumably being more naïve, exhibited a

less differentiated predation pattern, aligning with the findings of Mappes et al. (2014), where aposematism is more favourable during seasons with low numbers of naïve birds. In tropical habitats, such as our study locations, naïve birds are present throughout year (Stouffer et al., 2013) and not only during a few months of breeding period as in temperate regions. Thus, aposematic signals, coupled with chemical defences, effectively deter experienced predators but may attract attacks from naïve juveniles due to their conspicuousness (Mappes et al., 2014).

In laboratory experiments, it has been shown that birds can learn phenotypic cues associated to escape ability and change their predation pattern to avoid such unprofitable prey (Páez et al., 2021; Linke et al., 2022; Loeffler-Henry & Sherratt, 2024). Additionally, phenotypic cues commonly found in neotropical butterflies, such as dorsal iridescence, forewing bands or hindwing tails, might indicate difficulty of capture (Janzen et al., 2009; Pinheiro & Freitas, 2014; Linke et al., 2022). While testing avoidance due to evasive capabilities has seldom been tested with wild predators (Guerra et al., 2024), our results hint at some avoidance by immature birds. Nonetheless, attack avoidance of evasive prey might be higher in species less adept at catching flying insects (such as Cardinalidae or Thraupidae) but due to our low sample size per species and an unequal distribution between immatures and adults across habitats, this speculation remains to be tested in further experiments.

Targeting probability and capture avoidance: Although there are slight variations in capture rates between immature and adult birds, these differences are relatively minor. This suggests that all three prey phenotypes were equally susceptible to be captured when attacked, regardless of the birds' experience. This equal likelihood of capture could also be explained by the restricted space in our aviaries available for prey to escape capture, although we found that adults were slightly better in catching the evasive prey *Spicauda* (for a review, Wunderle, 1991).

Attack rates, on the other hand, diverge drastically between adult and immature birds. Adults preferred to target *Spicauda* and abstained from attacking *Heliconius*, while immature birds have shown remarkably equal attacking rates towards all three prey phenotypes. Learning and memorizing aposematic cues of unprofitable prey have been shown in behavioural laboratory experiments (Gibson, 1974, 1980; Skelhorn & Rowe, 2006; Hansen et al., 2010; Páez et al., 2021; Zvereva & Kozlov, 2021; Linke et al., 2022), including tests for birds of different age classes (Langham, 2006; Veselý et al., 2017). Predatorial behaviours observed in wild birds were not assessed using age groups (Guerra et al., 2024), or only used adults and a limited number of birds (Pinheiro & Campos, 2019). Thus, to our knowledge, conclusive evidence from the wild was scarce showing that avian predators differ in their prey attack preferences based on their age, although this is expected. However, since precise age determination is not feasible for wild birds, immature individuals from this study encompass a spectrum of experiences, ranging from recently fledged individuals to residents in the study locations of up to two years. Consequently, it is plausible that the immatures attacking *Heliconius* may be among the youngest.

Contrary to our expectations, *Spicauda* was not less likely to be caught by insectivorous birds, not even immature ones. Despite this, the summed instances of failed attacks are considerably higher for *Spicauda* compared to *Heliconius* or Euptychiines, pointing towards a bigger struggle for the birds to catch and subjugate *Spicauda* (Figure 5(c)). Food deprivation of birds prior to the experiment and the limited space in the aviary experiment, might have increased the mortality of *Spicauda* compared to natural conditions, as in nature unsuccessful attacks would be less likely to be followed by more attempts. During our video revision, *Spicauda* was often attacked repeatedly and unsuccessfully, managing to escape from attack multiple times. Conversely, attack behaviour towards *Heliconius* or Euptychiines was usually extremely fast and effective.

Evasive species usually show a high flying speed, which is correlated to high wing loading (Le Roy et al., 2019) and possibly driven by increased mass. Thus, such evasive species might be still profitable to chase by agile predators, while less agile or naïve predators might struggle to catch and subjugate the evasive prey.

Probability to survive upon predator attack: Like capture rates, mortality rates were relatively even across different prey phenotypes and age groups. While log-likelihood tests detected some differences for immatures, they were completely absent for adults, hinting at *Heliconius* and *Spicauda* being less likely to be killed when attacked by immature birds. *Heliconius* would thus be rejected after initial attacks due to chemical defences, while *Spicauda* is harder to catch and subjugate and thus less likely to be killed by less skilled predators. This is at least partly in agreement with our initial prediction, as species with high evasive capabilities or chemical defences are less likely to be killed by predators, at least for immature birds. Additionally, it points towards predation pressure being more equally distributed in more diverse habitats, which has thoroughly been supported for aquatic habitats but less so for terrestrial or tropical ones (see for review Chang & Todd, 2023).

Conclusion

Our study focused on the interactions of wild predators and their co-occurring prey, provides strong evidence supporting the efficiency of crypsis in reducing prey detection, aposematism via Müllerian mimicry in deterring attacks by sight rejection, and evasive flight behaviours in minimizing subjugation by predators. Notably, we found that the life stage of predators (immatures and adults) significantly affects the fitness of antipredator defences, suggesting that

the species diversity and age structure in different habitats play an important role in shaping predation pressures.

Acknowledgments

We would like to thank the Gallusser-Ramirez family for offering their property to conduct the study and their invaluable help and advice during our experiments. We thank the student helpers without whom the processing of nearly 300 hours of video material would have been impossible: Tereza Látalová, Sara Turková, Eliška Macháňová and Barbora Bělovská. We would like to extent our gratitude towards Leonardo Ré Jorge for his help and advice in the statistical analysis, Mario Alejandro Marin for help in determining Euptychiines, Alena Sucháčková for her input during the manuscript preparation, and Peruvian authorities at the Servicio Nacional Forestal y de Fauna Silvestre – SERFOR (Ministry of Agriculture) and colleagues at the Departments of Ornithology and Entomology (Diana Silva and Gerardo Lamas) of the Natural History Museum, Universidad Nacional Mayor de San Marcos.

Authors contributions

DL – study planning, field investigation, data curation and evaluation, video processing, statistical analysis, writing original draft, review and editing; **JHM** – field investigation, review and editing; **VNPNE** – field investigation, review and editing; **PG** – video processing, writing and editing; **CRG** – field investigation; **LSS** – study planning, supervision, review and editing; **ME** – study planning, statistical analysis, review and editing; **PMM** – study planning, field investigation, funding acquisition, review and editing.

Funding

Funding was provided by the Junior GAČR grant (GJ20-18566Y) and the PPLZ program of the Czech Academy of Sciences (fellowship grant L20096195) as well as GAJU n.014/2022/P. Logistic support in Peru was supported by 421 Fundación San Marcos, Letty Salinas was partially funded by Universidad Nacional Mayor de San Marcos RR 05557-R-22 (Project B22100321).

Birds were captured and used for experiments under the national permits D000088-2021-MIDAGRI-SERFOR-DGGSPFFS and D000047-2022-MIDAGRI-SERFOR-DGGSPFFS-DGSPFS. Butterflies were collected under the same national permits and RDG N.° 0488-2019-MINAGRI-SERFOR-DGGSPFFS and exported under the permits 003735-SERFOR and 003758-SERFOR.

Competing interests

We declare no competing interests.

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Table 1: Likelihood-based model tests of scenarios depicting the effectiveness of antipredator defences by three butterfly prey along the predation sequence. Akaike weights for the total number of birds and bird age (immature vs. adult) are shown. Best models are marked in ***bold and underlined****, while competing models (ΔAICc < 2) are only* ***bold****. Additional results for seasons and habitats can be found in Supplement 4.*

Stage	**Model**	**All different**	**All equal**	***Heliconius* different**	***Spicauda* different**	**Euptychiina different**
(1) Remaining undetected	Total	**0.47**	0.00	0.02	0.00	**0.51**
	Immature	**0.67**	0.00	**0.25**	0.00	0.08
	Adult	**0.22**	**0.11**	0.04	**0.31**	**0.32**
(2) Rejection upon identification	Total	**0.50**	0.00	**0.50**	0.00	0.00
	Immature	**0.44**	0.00	**0.56**	0.00	0.00
	Adult	**0.24**	0.06	**0.53**	0.03	0.13
(3.1) Targeting probability	Total	**0.25**	0.04	0.07	**0.62**	0.02
	Immature	0.10	**0.32**	**0.14**	**0.29**	**0.16**
	Adult	**0.36**	0.02	0.15	**0.47**	0.01
(3.2) Capture avoidance	Total	0.09	**0.37**	**0.16**	**0.14**	**0.24**
	Immature	0.08	**0.39**	**0.16**	0.14	**0.22**
	Adult	0.05	**0.46**	0.15	0.16	**0.17**
(4) Surviving attack	Total	0.06	**0.43**	**0.16**	**0.16**	**0.19**
	Immature	0.07	**0.40**	0.15	**0.16**	**0.22**
	Adult	0.05	**0.47**	0.15	0.16	0.16

Figure 1: Examples of butterflies in their native environment, used in the aviary experiments to assess responses of birds to different anti-predation strategies, from left to right: the cryptic Cissia penelope (Fabricius, 1775), the aposematic Heliconius erato (Linnaeus, 1758) and the evasive Spicauda simplicius (Stoll, 1790) (photos not scaled).

Figure 2: Stages of the predator-prey-interactions observed during the experiment, including a dichotomous flowchart depicting the progression of birds through the predation sequence.

Figure 3: Comparison of predation outcomes (no interaction, sight rejected, attacked, caught or killed) depending on the butterfly type (Euptychiines, Heliconius and Spicauda) and depending on the age of experimental birds (adult and immature).; ***(a)*** *distribution for all immature birds,* ***(b)*** *distribution for all adult birds and* ***(c)*** *distribution of all predator-prey interactions including all valid experiments. Plots for season and habitat can be found in Supplement 7.*

Figure 4: Stratum preference of birds and butterflies. ***(a)*** *Percentage of time spent (occupancy) by birds in the bottom, 0–200cm and the top, 200–400cm, of the aviary during the experiment (60 minutes). In the graph, only bird families with at least 5 valid experiments are presented. Drawing of birds are taken from Egg et al. (2010) and in order of panel A; from left to right: Myrmotherula axillaris, Habia rubica, Catharus ustulatus, Xenops minutus, Pitangus sulphuratus, Mionectes oleaginous, Thraupis episcopus and Vireo olivaceus. Bird pictures are scaled approximately.* ***(b)*** *Percentage of time spent by the three prey phenotypes (Euptychiines, Heliconius and Spicauda) at the two different heights in the aviary.*

Figure 5: ***(a)*** *Shared occupancy across high and low stratum in the aviary for all three used butterfly types. Compact letters denote significant differences between groups ($p<0.05$) based on Wilcoxon signed-rank test.* ***(b)****: Sum of instances of attacking behaviour without capture and*

capturing but subsequently losing the prey for each butterfly phenotype. ***(c)****: Duration until the first attack occurred for this butterfly type within the 60-minute experiment. Only for birds attacking at least one butterfly. Compact letters denote significant differences between butterfly types (Wilcoxon signed-rank test).*

Figure 1:

Figure 2:

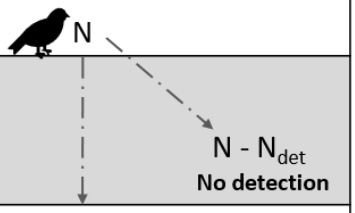
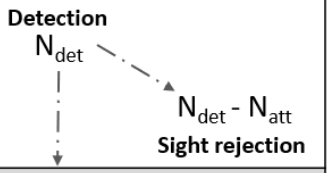
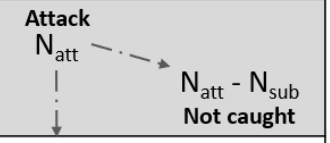
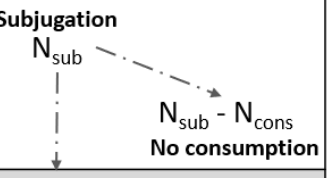
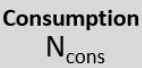
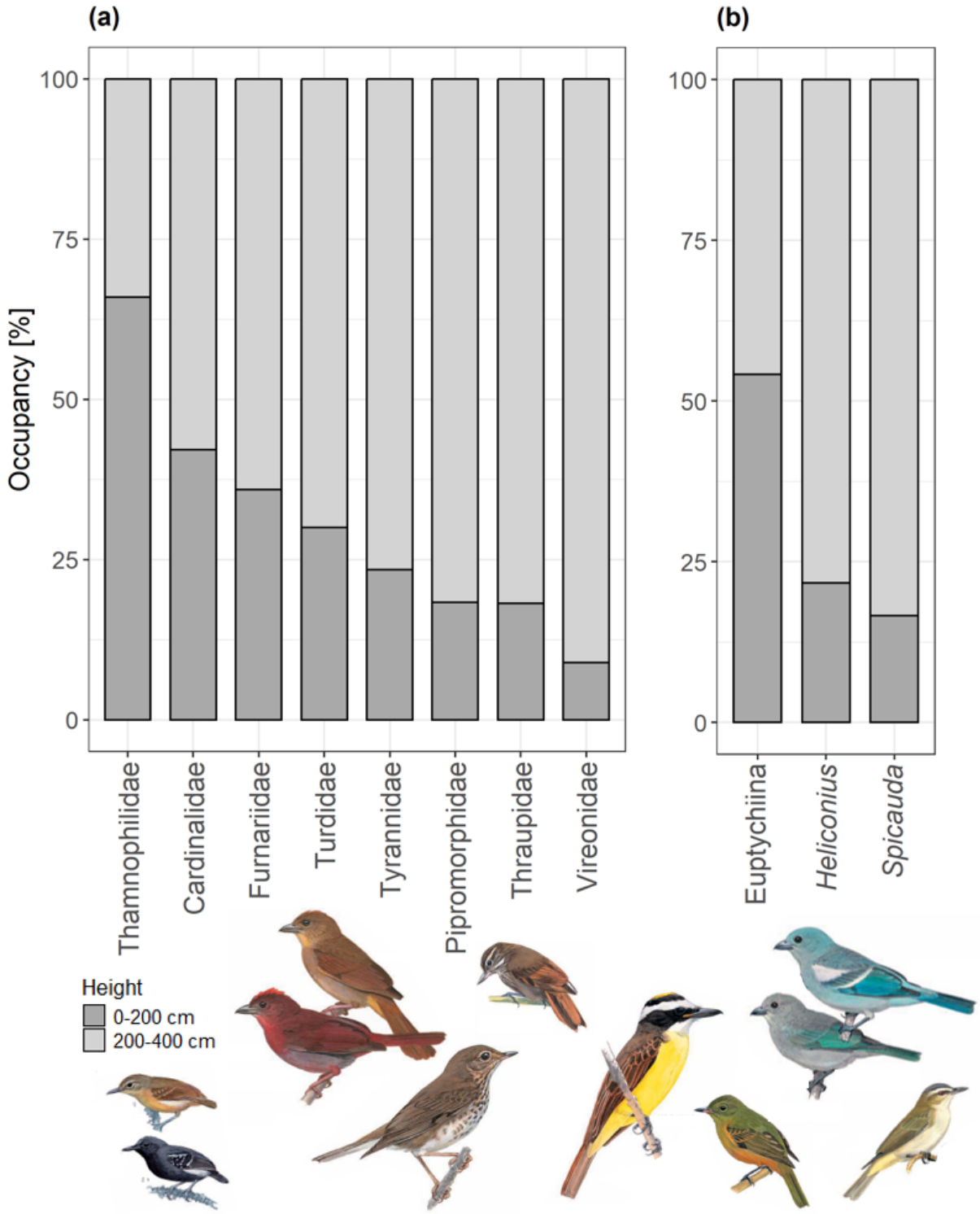
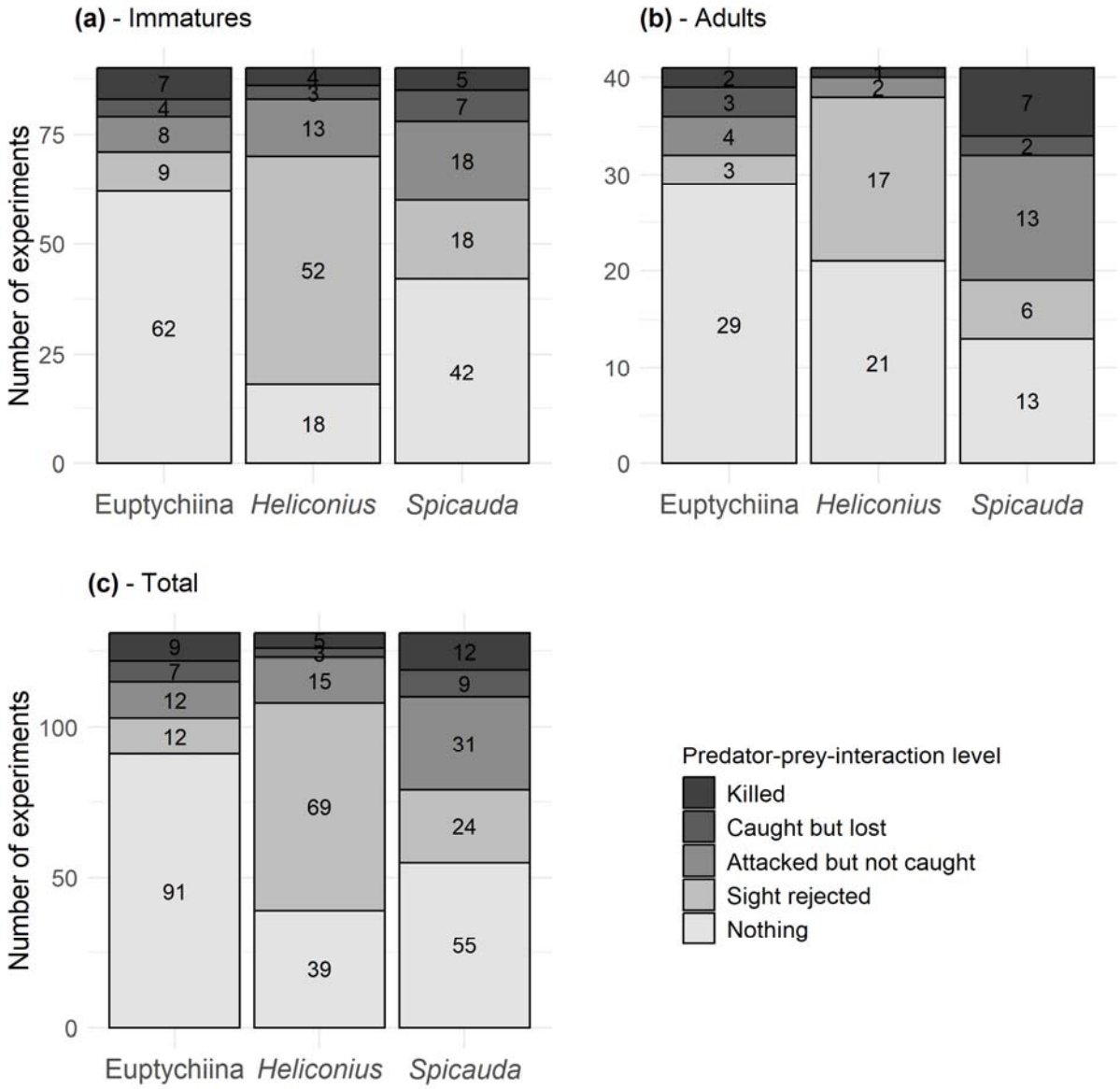
Parameter	Explanation	
Stage 1: **Encounter**	There was no visible interaction between the bird and the butterfly, and they stayed far apart during the whole experiment (never closer than ~ 20 cm). Likely no interaction due to spatial separation or crypsis.	
Stage 2: **Detection**	The bird was ~10 cm close to the butterfly and did not engage in pursuing, which suggests, that the first stages in the predation sequence, encounter and detection, were passed. But the bird deliberately choose not to pursue in any attacking behaviour.	
Stage 3: **Attack**	The bird engaged in pursuing behaviour towards the butterfly. The prey was identified as profitable and thus attacked, all attacking behaviour is counted (successful or unsuccessful).	
Stage 4: **Subjugation**	The bird successfully caught the butterfly with its beak. These events can lead to the consumption of butterfly as well as the release of it, for example, due to foul tasting or inexperience in the handling of this prey.	
Stage 5: **Consumption**	The butterfly was consumed by the bird. Attacking lead to capture. Killing and consumption of the butterfly, thus the prey was not rejected after killing.	

Figure 3:


(a)
(b)
Occupancy [%]
100
75
50
25
0
Thamnophilidae
Cardinalidae
Furnariidae
Turdidae
Tyrannidae
Pipromorphidae
Thraupidae
Vireonidae
Euptychiina
Heliconius
Spicauda
Height
0-200 cm
200-400 cm

705 Figure 4:



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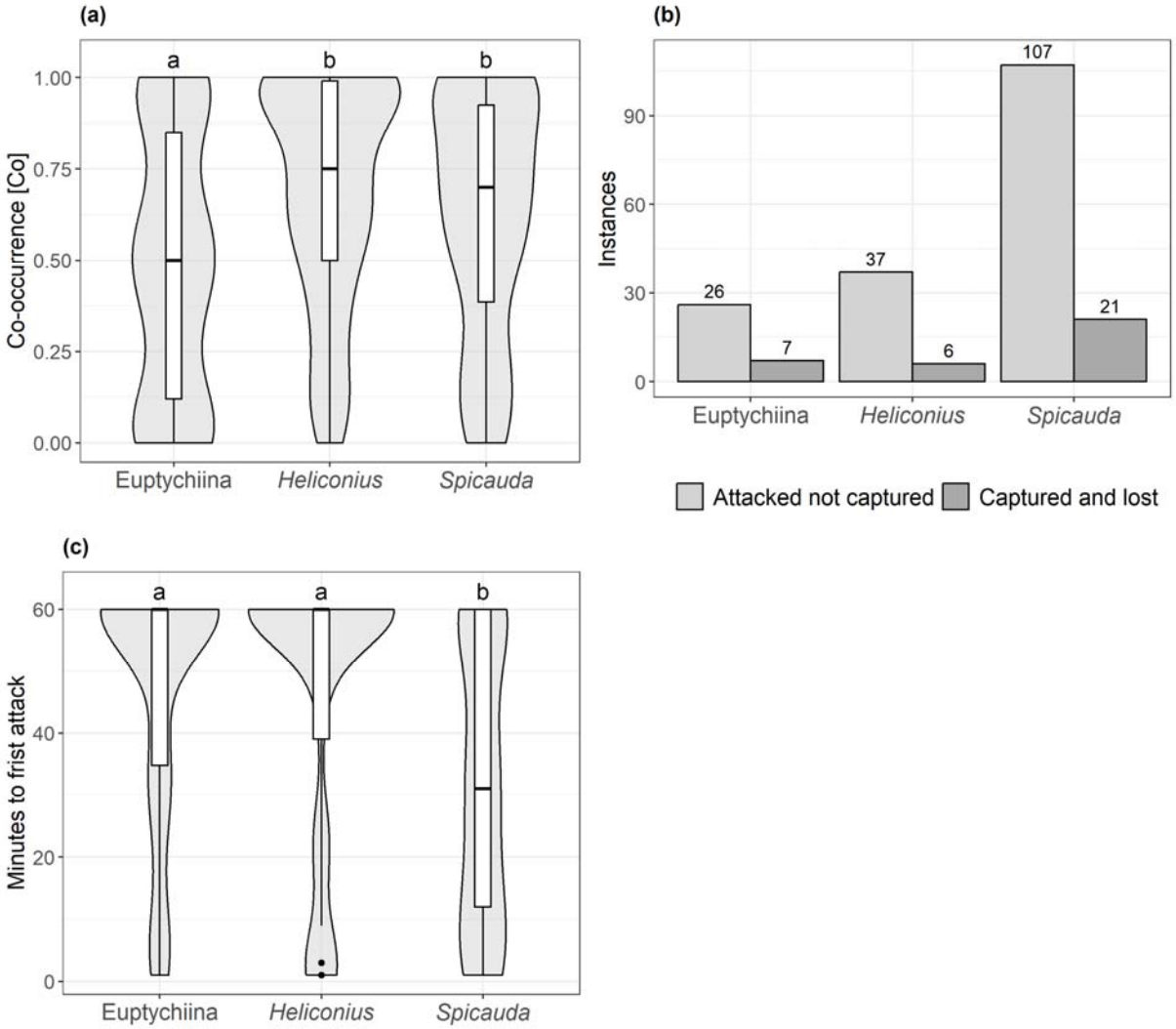
707 Figure 5:



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