**Foraging, fear and behavioural reaction norms – a lesson from hummingbirds**

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**Abstract**

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**Keywords**

**Introduction**

It is now widely accepted that animal’s foraging strategy (i.e. resource exploitation) depends not only on the amount and distribution of available food resources (Morrison et al. 1990; Herborn et al. 2014), and animal motivation (both in the sense of marginal value theorem (Charnov 1976) and/or body condition (Bautista et al. 2001)) but also predation pressure (Lima and Bednekoff 1999). The intensity of a prey vigilance increases with the level of risk predation (proportion/probability of predator presence), affecting prey foraging efficiency. As the risk allocation hypothesis (RAH) imposes, a prey allocates time for foraging inversely proportionately to predation pressure (Lima and Bednekoff 1999; Verdolin 2006). In the light of such a time trade-off, a fixed foraging strategy is expected to evolve, an adaptive behavioural norm in a given food-resources – predation landscape (Krebs 1980). However, numerous studies have demonstrated a high inter-individual variation in the foraging strategy e.g. (Patrick et al. 2014; Camprasse et al. 2017; Moldoff and Westneat 2017).

Recently, animals behavioural plasticity has been pointed out as an important source of the inter-individual variation in performance of any strategy, including foraging decisions (Nussey et al. 2007; Bell et al. 2009; Dingemanse et al. 2009; Herborn et al. 2014; Alonzo 2015; Toscano et al. 2016). Increasing evidence suggests that individuals do differ in their average level of behaviour displayed across a range of contexts (Carere and Maestripieri 2013) and in their responsiveness to environmental variation (Nussey et al. 2007; Dingemanse et al. 2009). Within a species/population individuals usually represent a full continuum of a given behaviour display, with two polar opposite phenotypes (e.g. high and low exploratory) and various intermediate forms in between. Importantly, fitness advantages of the contrasting behavioural phenotypes differ in various context, sometimes dramatically. For example, highly exploratory behaviour may favour an individual in inter-individual competition but it may also be disadvantageous in conditions of high predation pressure (Dall et al. 2004; Réale et al. 2007; Smith and Blumstein 2008; Quinn et al. 2012).

How behavioural variability is maintained in the population if selection favours a single optimal response remains an open question. This is particularly intriguing in the context of the risk allocation hypothesis (Lima and Bednekoff 1999) where, in given conditions of food resources availability and predation risk, a fixed behaviour is expected to provide the highest fitness across all possible scenarios. This apparent paradox can be solved by examining the fitness consequences of various behavioural performance during foraging in a gradient of risk predation. Here, we investigated fitness consequences (expressed by foraging efficiency) in regard to three behavioural traits: exploration (number of feeders used during the foraging visit), risk-avoidance (latency to approach the feeder to forage), and arousal (amount of movement during the foraging visit) in the context of varying level of perceived predation risk, in wild ranging, long-billed hermit hummingbirds (LBH, *Phaethornis longirostris*).

Hummingbirds are known for their extreme metabolism, and high need for energy intake that makes them constantly motivated to forage (Suarez 1992). Hence, hummingbirds are expected to exhibit high foraging efficiency regardless of the risk levels. This is particularly significant for traplining foragers, which use flowers dispersed across the habitat in a route-like fashion, a more unpredictable resource that has favoured behavioural traits that improve foraging efficiency (Araya-Salas t al 2018). On the other hand, such a free foraging foraging entails a period of high vulnerability for hummingbirds, which is regularly exploited by a wide range of predators (Stiles 1978; Owen and Cokendolpher 2006; Lorenz 2007; Zenzal et al. 2013; Sazima 2015; Nyffeler et al. 2017). Thus, a trade-off between foraging and avoiding predation is expected to be particularly pronounced in hummingbirds. This obviously hinders *a priori* prediction for birds response to conditions of varying predation risk. We explored the trade-off between resource explotation efficiency and risk avoidance by examining individual variation on behavioral traits expected to affect foraging efficiency and their interplay with varying levels of risk. We expected that foraging efficiency will be positively correlated with exploratory behaviour. This is because exploratory individuals, often being more experienced as well as bolder in terms of risk-aversion (Carere and Maestripieri 2013) are likely to ignore somehow novel items, and onset foraging faster, compared to less exploratory individuals. For both risk-avoidance and arousal we expected a negative relationship with foraging efficiency. The two traits potentially increase time allocation for activities not related with foraging *per se*, so they are also likely to decrease foraging efficiency per unit of time. Finally, for each trait we measured its repeatability (Bell et al. 2009), thus evaluated them as personality traits (Dingemanse et al. 2009).

**Material and methods**

*Fieldwork.* We carried out the study at the area of one of LBH leks in La Selva Biological Station, Costa Rica (10 o23’ N, 84o10’ W) between May and June 2015. Birds were individually marked [foam tags of unique colour combinations, attached to bird back and breast with nontoxic eyelash glue, see Araya-Salas et al. 2018 for details] and habituated to use feeders. Of the XX birds marked at the study lek (XX territorials and XX floaters or females), 12 individuals regularly used feeders utilized in the present study (located at XX distance from the closest lek border) and so were tested in the experiment.

To quantify birds behaviour in various risk predation context, we applied a field experiment using a three one-flower feeders for the experimental set up. We arrange the feeders in a line, separated by ca 10 cm distance from each other. The experiment consisted of two phases – hereafter control and experimental. Both phases were performed at the same day, with the control being directly followed by the experimental phase. The experimental phase started at the moment when all the focal individuals have been recorded at the feeder for the control conditions, and lasted until all of them visited the feeder again in the experimental conditions. Since focal individuals visited feeders regularly, appearing at the feeders site on average XX times per hour, we were able to complete the two phases within ca XX hours. During both phases of the experiment the three feeders were filled up with ~ 30% sugar-water and birds were allowed to forage on the nectar spontaneously, while their behaviour at feeders site were recorded by human observer and a commercial camera (continuous recording mode). The only difference between the control and experimental phase were a threat model attached to feeders during the experimental phase. For the threat model, we used specimens of the bullet ant (*Paraponera clavata*; found dead in the forest). Although the ant is not a predator of hummingbirds (including LBH), the ant-bird encounter imposes potential danger for the bird in the form of being bitten. Indeed, an average birds response for the threat exposure was as expected (see results). We performed total of four complete control-experimental sessions within ca two weeks.

We first screened video recordings with VLC software (www.videolan.org), to locate and cut out video fragments with foraging visits of all focal birds. As a foraging visit we considered birds’ visit at the feeder area when it inserted the bill into a feeder at least once. For each visit (both at control and experimental phase) we established several crucial time-points (white and black circles on Fig. 1), with 0.1 seconds precision, using Cowlog software (Pastell 2016). Based on these time-points we calculated duration of events such as: latency to forage [the interval between appearance in the feeder area (usually hovering in front of the feeder) and the onset of foraging, i.e. distance a-b on Fig. 3]; foraging interval/s (e.g. distance b-c, Fig. 3; on average XX events); foraging break/s (e.g. distance c-d, Fig. 3; on average XX events); total foraging (distance b-e, Fig. 3) and total foraging visit (distance a-e, Fig. 3). For each visit we also noted which and how many times each of the three feeders were used by the focal bird.

To further analyse birds behaviour we calculated three functional behaviours, likely to be related to three personality traits: exploration, risk-avoidance and arousal (Réale et al. 2007). As a proxy for **exploratory behaviour** we utilized a **standardized number of feeders** - totalnumber of various feeders (i.e. 1-3 feeders) used during the foraging visit divided by the total foraging duration. As a proxy for **risk-avoidance** we used **latency to approach the feeder** – as defined above (latency to forage, distance a-b on Fig. 3). For both the parameters, we assumed that the higher the value, the stronger is the exhibited behaviour.

As **arousal** we considered a total amount/range of movements at the feeder recorded during the total foraging time (distance b-e on Fig. 3), standardized by the number of feeder changes. For that we first processed the video recordings using Tracker software (physlets.org/tracker), which allowed to establish Cartesian coordinates of a bird position for each video frame. Then, we calculated coefficient of variance of bird movements based on the formula: sqrt((xj - xi)2 + (yj - yi)2), where xi,j and yi,j are Cartesian coordinates of the bird position in a focal i and previous j video frame. Since the birds movements could be biased by exploratory behaviour, we standardized this coefficient dividing it by the number of feeder changes at given visit. We expected that the higher value of the coefficient, the higher arousal.

Finally, we calculated **foraging efficiency**, which was the ratio of total duration of foraging (sum of all feeding intervals) to the and duration of the foraging visit.

*Data analysis.*

All the analyses were performed in R environment (R Core Team 2017). We compared foraging efficiency between control and experimental conditions using linear mixed models (LMM; Zuur et al. 2009), with treatment as a fixed factor. To address the issue of pseudoreplication (related to multiple testing of the same individuals) we included bird’s identity as a random factor in the model. To test an effect of individual performance, we compared model with and without bird identity (*Chisq* test). For examining repeatability of individual’s behaviours we also applied modelling approach (Nakagawa and Schielzeth 2010) using *rptR* package (Stoffel et al. 2017). Then, we analysed foraging efficiency in regard to each behaviour separately, again using modelling approach (LMM), with treatment being a fixed factor and bird identity as a random factor.

**Results**

Foraging efficiency was strongly affected by the experimental treatment (LMM, estimate = -0.11, SE = 0.03, t = -3.91, df = 188.17, P < 0.001), in general being lower in experimental conditions (Fig 2A). There were also individual differences in foraging efficiency in response to the treatment (Chisq = 17.06, df = 1, P < 0.001), with some individuals being more efficient in control conditions while others exhibiting a reverse pattern; in one case foraging efficiency was similar in both control and experimental conditions (Fig. 2B).

**Repeatability** of behaviours was moderate and significant for exploration (R = 0.28, CI = [0.044, 0.475]; LRT: P < 0.001) and risk-avoidance (R = 0.269; CI = [0.064, 0.454], P < 0.001), and very low and insignificant for the arousal (R = 0.007, CI = [0, 0.09]; LRT: P ~ 1).

Overall, intensity of the **exploratory behaviour** (i.e. number of feeders used during the foraging visit) was related to foraging efficiency (LMM, estimate = 0.47, SE = 0.18, t = 2.53, df = 192.95, p = 0.01) though similar in the two treatment (LMM, estimate = 0.09, SE = 0.04, t = 2.12, df = 186.57, P < 0.04). Nevertheless, direction of the relationship between the number of feeders used and foraging efficiency was clearly condition-dependent (as indicated by the treatment x explorative behaviour interaction, LMM, t = -5.72 , df = 188.5, p < 0.001). It was positive in control conditions (LMM, estimate = 0.50, SE = 0.16, t = 3.18, df = 139.21, P = 0.002), and negative in threatening conditions (LMM, estimate = -0.93, SE = 0.21, t = -4.36, df = 49.87, P < 0.001 Fig. 3A).

Latency to approach a feeder (**risk-avoidance**) was affected by experimental treatment (LMM, estimate = -0.13, SE = 0.04, t = -3.16, df = 191.33, P = 0.002; Fig. 5B). Overall, and independently on conditions, foraging efficiency decreased with the duration of the latency (LMM, estimate = -0.02, SE = 0.01, t = -2.19 , df = 191.99, P = 0.03; interaction of latency and treatment: estimate = 0.02, SE = 0.01, t = 1.53, df = 191.99, P = 0.13; Fig. 3B).

The range of birds movements (**arousal**) was positively related to foraging efficiency (LMM, estimate = 0.04, SE = 0.01, t = 3.85, df = 188.55, P < 0.001), and overall higher in the threatening conditions (LMM, estimate = -0.33, SE = 0.05, t = -6.13, df = 188.71, P < 0.001). The relationship between the foraging efficiency and arousal was also stronger in the experimental conditions (as indicated by the treatment x exploratory behaviour interaction, LMM, t = 5.23, df = 188.36, p < 0.001; Fig. 3C).

**Discussion**

As we expected in the light of the risk allocation hypothesis (XXX), foraging efficiency of the LBH decreased in response to threatening conditions. However, behavioural performance of individuals in regard to exploration, risk-avoidance and arousal additionally affected the foraging efficiency, interestingly in condition-dependent manner. These results demonstrate that a range instead of a single, fixed strategy should be considered, when modelling the time allocation into foraging in the context of predation pressure. The results also highlight the importance of behavioural variability in shaping evolution of foraging strategy.

Despite not being a specific target of any predator, hummingbirds may be opportunistically hunted by a wide range of predators (e.g. Owen and Cokendolpher 2006; Lorenz 2007; Zenzal et al. 2013; Sazima 2015; Nyffeler et al. 2017). That imposes a considerable predation risk and favours evolution of risk-avoidance behaviour. Indeed, we found that experimental exposure of an ant, that potentially represents just a threat of being bitten (but not eaten), was enough for LBHs to exhibit higher risk-avoidance behaviour compared to the control conditions (i.e. expressed by the intercept of the regression lines between the risk-avoidance and foraging efficiency in the control and experimental conditions; Fig. 3B). Importantly, despite of the threat birds did forage, although with a lower efficiency. This places LBHs (and potentially all trap-line foraging hummingbirds) in the group of species being example of so call paradox risk-allocation hypothesis (RAH; Lima and Bednekoff 1999; Ferrari et al. 2009). XXXXX.

3. Context dependent foraging efficiency and behavioural components of the foraging performance.

Zachowanie eksploracyjne zwiększało wydajnośc żerowania w warunkach kontrolnych, natomiast zmniejszało je w warunkach eksperymentalnych. To pierwxsze lekko dziwi, bo eksploracja czasem odbywa się kosztem efektywności (XXXX). Ale być może klucz polega na tym, że taki zmieniający często feedery jest tez po prostu wyżej zmotywowany, zre lepiej, a potem zmieniajac miejsce to tak naprawdę rozwaza koniec, ale potem nie umie sobie odmówić. To drugie jest już bardziej zrozumiale – jak często zmienia kwiatek za każdym razem musi niejako na nowo rozwazyc niebezpieczeństwo i to wpływa na jego efektywność zerowania. Te przeciwstawne odpowiedzi podkreślają jak rozne może być dostosowanie wynikające z określonego zachowania zaleznie od kontekstu!!! To sugeruje, ze plastyczność może być efektem ewolucji, a to z kolei, mówi dużo o tym jak utzymywana jest taka różnorodniosc w populacji mimo silnego parcia na ewolucję pojedynczej strategii.

Być może to jest dalekoidąca spekulacja ale to oznacza, ze traplinersy mają w sumie mniejsza efektywność zerowania niż terrytorialsy, bo nie znając otoczenia i często zmieniając kwiaty wiele czasu traca na rozpoznanie terenu.

Latency generalnie obniza wydajność, czyli im strachliwszy tym się mniej naje. To może być potencjalnie mechanizm adaptacyjny poddtrzymujacy róznordbosc behawioralna – jak ktoś strachliwe będą nagradzane przyzyciem, a te niestrachliwe nazarciem się, obie formy mogą w pewnych warnkach przynosci dobre dostosowanie. To tez pokazuje jak może być ta roznorodnosc poddtrzymywana

Arousal – im wiekszy tym wieksza efektywnosc – co może być związane z predkoscia podejmowania decyzji, ale to nie jest zbyt dobra cecha na cokolwiek, w tym jest mało powtarzalna jako personality

4. Examined behaviours as personality traits

Oceniajac powtarzalnosc dla warunkow kontrolnych epolration and risk avoidance uzyskały względnie wysokie notowania. Arousal nie bardzo, awiec nie może być rozważany jako cecha personality. Te dwie pozostałe być może są, choć to oczywiście jest osobne pytanie , np. czy sa dziedziczne. Poza tym pewien problem jest taki, że across the context one nie są konsistentne, ale być może nie powinno się oczekiwać takiej consistency – bo być może odpowiedz po prostu jest rozna zaleznie od kons

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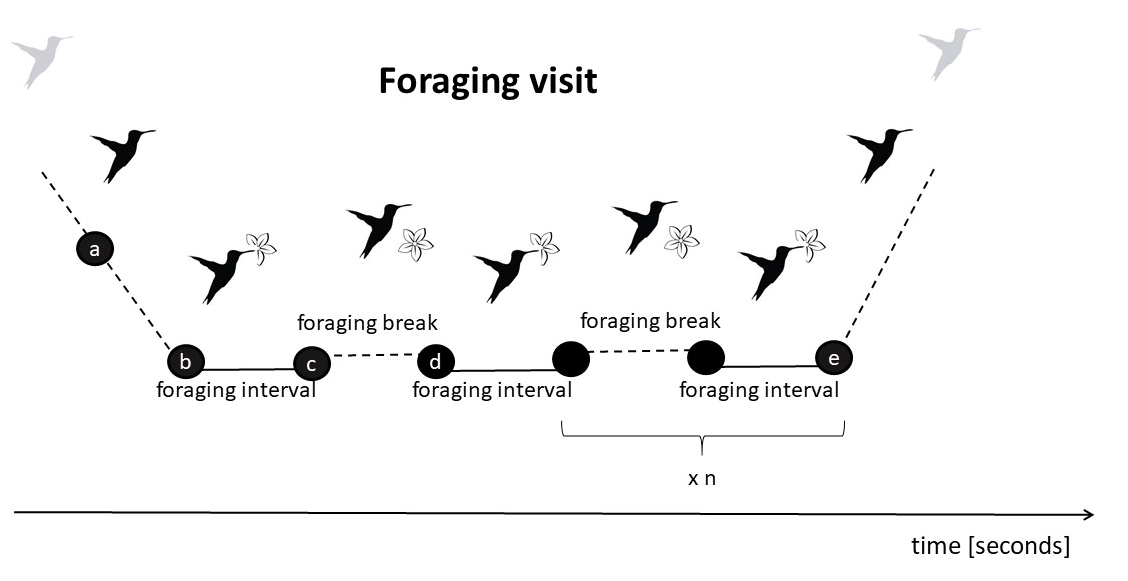
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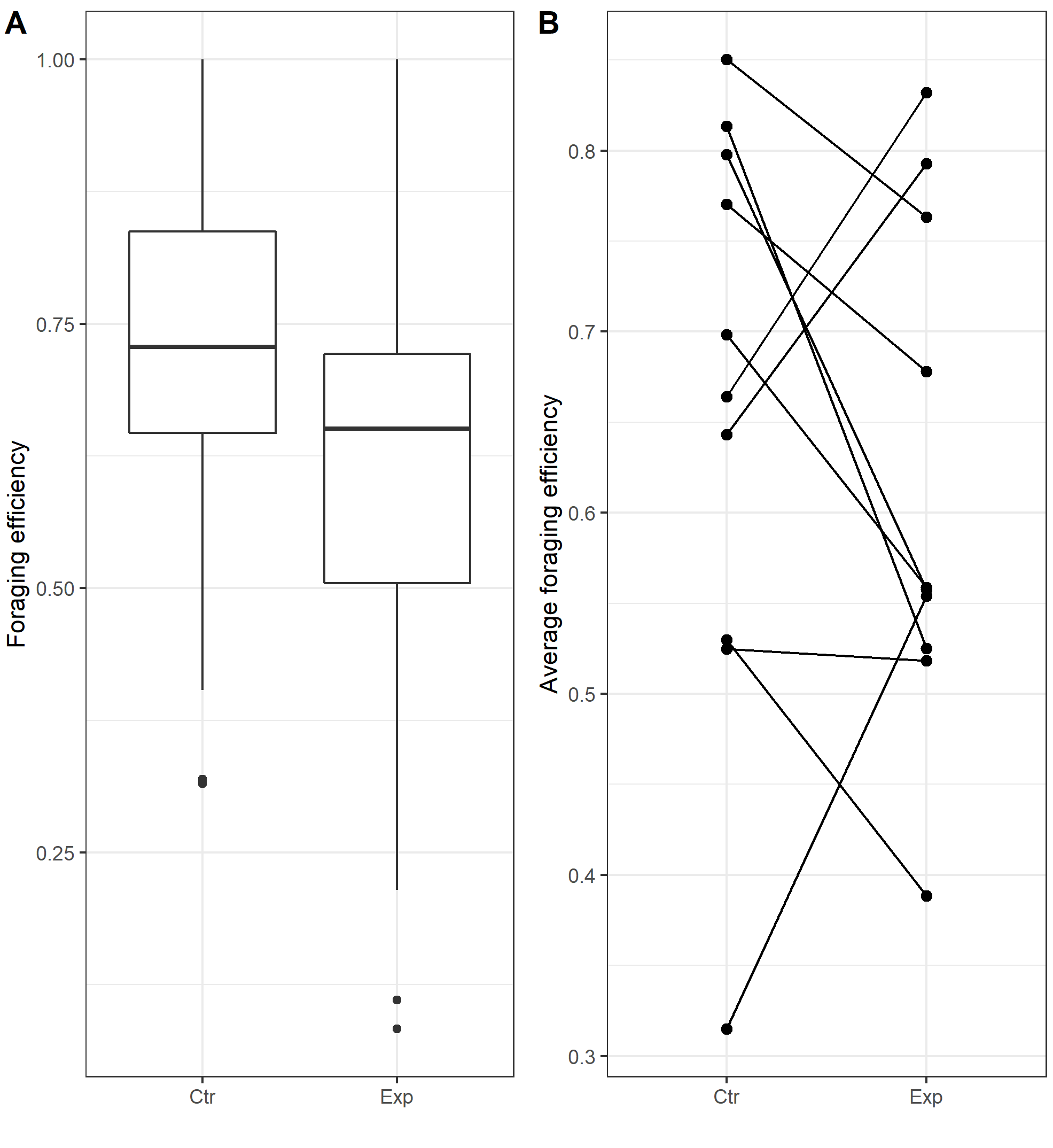
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**Figure 1.** Components of the foraging visit.



**Figure 2.** Average foraging efficiency of each focal individual in the context of low (control) and high (experimental) levels of perceived risk of predation.



**Figure 3.** Foraging efficiency of long billed hermits in regard to their behavioural performance in the context of low (control - Ctr) and high (experimental - Exp) levels of perceived risk of predation.

