

# Impacts of nectar robbing on the foraging ecology of a territorial hummingbird

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## ABSTRACT

While the effects of nectar robbing on plants are relatively well-studied, its impacts from the perspective of the pollinators of robbed plants is not. Numerous studies do consider the impacts of robbing on pollinator visitation to robbed plants, but rarely do they focus on its scaled-up impacts on individual pollinator behavior. We used radio telemetry to track the spatial and behavioral responses of the territorial hummingbird *Aglaeactis cupripennis* to experimental nectar robbing over a period of several days. Simulated nectar robbing impacted foraging behavior by increasing territory area, distance flown, and reliance on novel food resources, especially small-bodied flying insects. We did not observe any impact on the amount of time individuals spent foraging, nor did we observe territory abandonment. These findings indicate that nectar robbing may impose a significant energetic cost on pollinators via increased flight distances and shifts towards potentially less profitable food resources, and demonstrate the importance of quantifying the indirect effects of nectar robbing on pollinators in addition to plants.

## 1. Introduction

Plant-animal mutualisms can impact the population structure (Abe et al., 2011; Pérez-Méndez et al., 2016), gene flow and reproduction (Carlo and Morales 2016; Sork et al., 2015), and community assembly and evolution (Bartomeus et al., 2016; Carstensen, 2016; Song and Feldman, 2014) of the plants and animals involved. Mutualisms can be impacted indirectly in critical ways by the effects of ecological interactions with species outside of the mutualism. Nectar robbing is a common and widespread cause of indirect effects in plant-pollinator mutualisms (Irwin et al., 2010). Nectar robbing occurs when an animal extracts nectar from a flower but bypasses the reproductive structures, usually by means of a small incision in the flower corolla (Inouye, 1980; Maloof and Inouye, 2000).

Nectar robbing has been shown to have a range of direct and indirect effects on plant reproduction, ranging from positive (Navarro, 2000; Singh et al., 2014; Waser, 1979) to neutral (Arizmendi et al., 1996; Hazlehurst and Karubian, 2016; Zimmerman and Cook, 1985) to negative (González-Gómez and Valdivia, 2015; Irwin and Brody, 1999; Roubik, 1982). Several factors determine what the strength and direction of robbing will be on plant fitness, including pollen limitation and plant mating system (Burkle et al., 2007; Irwin et al., 2010) and whether robbers destroy floral reproductive structures during foraging

(McDade and Kinsman, 1980).

In contrast to the rich body of literature that exists for plants, the effects of nectar robbing on pollinators is sparse (Irwin et al., 2010). From the animal's perspective, changes to a plant-animal mutualism often involve changes in resource availability, such as decreases in fruit or nectar associated with selective harvesting of food sources. In some plants, nectar robbing can lead to continued nectar production but evaporation of nectar through the robbing incision in the side of the flower. This evaporation can lead to chronically lower nectar volumes and also concentrates the remaining nectar, which substantially increases its viscosity and can make it difficult for pollinators to consume (Pleasants, 1983; Zimmerman, 1988). Pollinators may respond to the decrease in nectar availability and increased difficulty of nectar extraction by avoiding robbed flowers (González-Gómez and Valdivia, 2015), with consequences for plant fitness (Irwin and Brody, 1998; Pyke, 1982). To our knowledge, no previous studies have observed if these one-time avoidance responses by pollinators to robbed flowers scale up to impact the overall foraging ecology of individual pollinators.

There exists an extensive literature on animal responses to changes in resource distribution in other contexts (Gray et al., 2002; Simon, 1975), which suggests that animals exhibit flexible behavioural responses to changes in resource availability. In the case of territorial species, both optimal foraging theory and empirical studies suggest that

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territoriality can only be maintained if the benefits of exclusive access to a resource patch outweigh the costs (Gill and Wolf 1975; González-Gómez et al., 2011; Trombulak, 1990), and animals adapt to resource depletion to mediate this interchange. For example, during times of resource depletion individuals may continue to defend territories but expand their home ranges, presumably to gain access to sufficient resources to maintain their energy intake (Carpenter, 1983; Edwards et al., 2013; Hixon et al., 1983; Ruby et al., 1994). However, larger home ranges are more energetically expensive to forage in, and animals may need to compensate by spending less time and energy in territory defence (Powers and McKee, 1994). Territorial individuals may also respond to resource depletion by expanding their diet niches to include secondary food resources (Jedlicka et al., 2006; Pimm et al., 1985) or by devoting more time to foraging (Garrison and Gass, 1999; Temeles et al., 2005). Alternatively, if resources are depleted below a critical level on the territory, this may lead to territory abandonment and adoption of a “floater” foraging strategy (Brown and Sherry, 2008). Justino et al. (2012) observed territory abandonment by hummingbirds in response to experimental reductions in floral resources.

The degree to which animals match their foraging strategy to changes in resource reduction is likely to have important effects on net energy gain and fitness (Suarez and Gass, 2002). In the case of pollinators, different foraging strategies may have significant effects on pollination services to plants (Maruyama et al., 2016). However, the ways in which pollinators may shift their foraging strategy in response to nectar robbing, and what the ramifications for animal energetics and demographics may be, is currently unclear. This is especially true when one considers the range of mutualism strengths exhibited in pollination networks, from highly specialized 1:1 interactions to more generalized interactions.

In systems where nectar robbing causes chronic nectar depletion it is possible that territorial pollinator species may abandon their territories (Justino et al., 2012) or switch to non-territorial foraging strategies. Shifts in foraging behaviour by pollinators may in turn have important consequences both for the energetics of the pollinator (Shackleton, 2016) as well as for gene flow in the plant (Maruyama et al., 2016). To fill this knowledge gap, we exposed the territorial hummingbird species *Aglaeactis cupripennis* to nectar robbing of its preferred food source, the Andean tree *Oreocallis grandiflora* (Family: Proteaceae) in the wild, and monitored their behavioural response in terms of territory area, distance flown, activity budgets, and diet. Hazlehurst and Karubian (2016) found that after simulated nectar robbing in *O. grandiflora* flowers were still able to produce nectar, however robbed flowers contained less nectar than unrobbed flowers. Nectar in robbed flowers also had a higher sucrose concentration than in unrobbed flowers, suggesting that evaporation of nectar occurs through the robbing incision and causes nectar sugars to become more concentrated. We therefore inferred that these responses would scale up to the territory level and have significant impacts on the foraging ecology of individual hummingbirds. Specifically, we hypothesized that focal *A. cupripennis* individuals would exhibit both territory expansion and an associated increase the time spent foraging. We did not expect to see diet shifts, due to the apparent tight mutualism between *A. cupripennis* and *O. grandiflora* and the relatively low nectar volume and sucrose content of other flowers within *A. cupripennis* territories as compared to *O. grandiflora*, and we did not expect to see territory abandonment due to the apparent limited availability of open territories at our study sites.

## 2. Materials and methods

### 2.1. Study site

This study took place from 2014 to 2015 in montane evergreen forests of Peru and Ecuador. Data were collected in 2014 in Peru between 2900–3100 m asl at the Wayqecha Biological Station in Manu

National Park, Cuzco Province (13°10′29″S, 71°35′14″W) and in 2015 in Ecuador between 2850–3100 m asl at the Bosque Comunal El Merced, Azuay Province (2°59′39″S, 78°44′01″W). The change in field site was precipitated by a lack of birds in Peru in 2015, perhaps due to El Niño conditions altering the poorly understood altitudinal migration patterns of *A. cupripennis*. Fieldwork in both sites corresponded to the transition from dry to rainy season and the start of the breeding season.

### 2.2. Study system

The shining sunbeam (*Aglaeactis cupripennis*) is a mid-sized hummingbird with a mean mass of 7.5 g that occurs in Andean cloud and montane forest and high-altitude grasslands from Southern Peru to Colombia at elevations from 2300–4300 m asl. Males are distinguishable from females by the extent of purple plumage on the back. Both sexes defend stands of Andean firebush (*Oreocallis grandiflora*; Family: Proteaceae), a shrub or small tree which produces terminal flowered raceme inflorescences of 10–50 tubular paired flowers that open sequentially from the base of the inflorescence towards the top. Hazlehurst et al. (2016) reported the standing crop and sucrose concentrations of *O. grandiflora* to be  $13.8 \pm 1.2 \mu\text{L}$  ( $n = 123$ ) and  $28.5 \pm 1.6\%$  Brix ( $n = 123$ ). Nectar robbing at both sites is caused by birds of the genus *Diglossa* (family: Thraupidae), including *D. cyanea*, *D. brunneiventris*, *D. mystacalis*, and *D. humeralis*. Mean natural robbing rates were measured by Hazlehurst and Karubian (2016) at  $21.0 \pm 0.3\%$  of flowers per inflorescence ( $n = 110$ ), but robbing intensity was observed as high as 100% of flowers per inflorescence in some parts of the field sites, especially along roads (J. Hazlehurst, personal observation).

### 2.3. Territory mapping

Hummingbirds were captured in stands of *O. grandiflora* using 30-mm mesh mist nets. Once a bird was captured, a 0.25 g radio telemetry tag (Blackburn Telemetry, Inc.) was attached to the back 1 cm below the intra-scapular region with eyelash glue (Fig. 1) (Hadley and Betts 2009). Antennas were trimmed dramatically to reduce any effects on flight and to further reduce the weight of the tag. Only adult birds for whom the weight of the tag was less than 3% were tagged (Kenward 2001), and no gravid females or obviously unhealthy or injured birds were tagged. Tags generally fell off naturally after 1–2 weeks – and on some occasions earlier. Additional data including mass, age, sex, and breeding status were also recorded. We placed tags on a total of 32 hummingbirds. Of these, 17 individuals were not used in the analyses below because they were either never located again after the initial tagging or had territories in areas where we could not track them due to dangerously steep terrain.

To map initial hummingbird territories, individuals were tracked for 8 h over a 2-day period, in alternating 2-h observation sessions in the morning and afternoon. Scan samples were conducted every 5 min to record coordinates and behaviour of the focal bird (See Supplementary information Table S1), and feeding observations were continuously recorded.

Kernel density analysis was conducted using the package ‘adehabitatHR’ (Calenge, 2006) in R version 3.2.3 (R Core Team, 2015) to map the 95th kernel of the territory. We used a rule-based method to minimize kernel area while restricting polygon fragmentation to calculate the smoothing parameter  $h$  (Kie 2013) using  $h_{ref}$  as a starting point. If the bird was known to have a distinctly segmented home range, we adjusted  $h$  to minimize both the number of biologically reasonable territory segments and the area of each segment.

### 2.4. Nectar robbing simulations

Each replicate of our experiment took place over five consecutive days beginning on the day after tag placement, unless weather



Fig. 1. (A) *A. cupripennis* with radio transmitter tag (B) *Diglossa* robbing flowers of *O. grandiflora*.

interfered, in which case we rescheduled as soon as possible. For the first two days of the experiment, hummingbird territories were mapped for both control and treatment birds using the methods described above; we refer to this as the “before” stage hereafter. On day 3, we robbed every accessible *O. grandiflora* flower within the calculated territory for robbed treatment individuals. We simulated nectar robbing on *O. grandiflora* by making a 0.5 cm incision approximately 0.75 cm above the calyx on the lateral edge of the corolla. A 20  $\mu$ L microcapillary tube was then inserted into the incision and all nectar present was removed. All flowers on the inflorescences that were likely to open during the experiment were robbed, even if not yet open. All nectar was removed because in preliminary aviary trials in which *Diglossa* nectar robbers were offered fresh *O. grandiflora* flowers, the birds consumed all the nectar in the flower (Hazlehurst and Karubian, 2016). Our robbing treatment did not permanently remove all nectar from the flowers, as Hazlehurst and Karubian (2016) found that flowers can continue to produce nectar after experimental robbing. However, Hazlehurst and Karubian (2016) also found that experimentally robbed flowers contained significantly less nectar than unrobbed flowers and at a higher sucrose concentration, suggesting continued nectar secretion but subsequent evaporation through the robbing incision. In control territories, we simulated the nectar robbing process on day 3, but did not actually rob flowers; for treatment territories, in all cases at least 90% of all *O. grandiflora* flowers were robbed. While our experimental nectar robbing levels were higher than the observed natural average, nectar robbing intensity is known to vary greatly over time (Irwin and Maloof, 2002) and thus natural robbing levels could theoretically approach our treatment.

On the last two days of the experiment, we continued the same tracking protocol for two more days; we refer to this as the “after” stage of the experiment. We also quantified available floral resources in each of the pre-robbing territories by walking two transects at 90° angles to one another across the pre-robbing 95th territory kernel. At every 10 m along transects we recorded a count of every flower known to be visited by hummingbirds within a 5 m radius, canopy height and percent cover. Insect resources were quantified by placing sticky fly tape at 5 random points within territories on 5-m posts. After 24 h tape was recovered and the number of insects on each piece was counted. We

opportunistically sampled different hummingbird flowers in territories for nectar volume and sucrose concentration and used this data in combination with flower density data from our transects as an estimate of territory quality by calculating the kilocalories available in hummingbird-visited flowers in each territory (Stiles, 1975).

## 2.5. Statistical analysis

All analyses were conducted in R version 3.2.3 (R Core Team, 2016). We calculated the minimum convex polygon (MCP) and the 95th kernel area (using coordinates from 5-min scans) with the ‘kernelUD’ function in the ‘adehabitatHR’ package (Calenge, 2006). To calculate the total distance flown, we used the function ‘as.ltraj’ in the package ‘adehabitatLT’ (Calenge, 2006) and then calculated the cumulative distance flown in the before and after treatment stages.

To analyse the effect of treatment (control or robbery) on the spatial foraging behaviour in terms of the MCP area, 95th kernel area, and the total distance flown, we used linear mixed models (LMM) in the function ‘lme’ in the package ‘nlme’ (Pinheiro et al., 2016). The following methods are common to all three models. The interaction of treatment (robbed vs. control) and stage (before or after treatment) was used as the fixed effect. Bird nested within site was used as the random effect to account for potential differences between sites and individuals. We initially included sex, breeding status, and kilocalories from nectar available in each territory as covariates, ran models with all possible combinations of these covariates, and dropped all non-significant covariates from the model. We then used corrected Akaike’s Information Criterion (AIC<sub>c</sub>) to select the best model from the remaining options. In cases where competing models were similar ( $\Delta$ AIC<sub>c</sub> < 2), we calculated marginal R<sup>2</sup> values using the function ‘r.squaredGLMM’ in the package ‘MuMIn’ (Barton, 2016). The final model for the MCP analysis included log-transformed MCP area as the dependent variable and breeding status as the only covariate. For the 95th kernel area analysis, the best model used the log-transformed area of the 95th kernel as the dependent variable and breeding status as the only covariate. The best model for the distance flown by *A. cupripennis* used the log transformed distance flown as the dependent variable and sex as a covariate. In all models we also included an offset for the number of observations per

individual. We then conducted post hoc, pairwise tests with a Bonferroni correction ( $\alpha = 0.0125$ ) to ascertain the degree and directionality of the difference between control and treatment individuals before and after treatment stages using the package ‘lsmeans’ (Lenth, 2016).

We used LMM to analyse the effect of treatment on the proportion of time focal individuals spent foraging, with the logit-transformed mean proportion (Warton and Hui, 2011) as the dependent variable, the interaction of stage and treatment as the fixed effects, and sex, breeding status, and the density of flowers and small-bodied flying insects as covariates, and an offset to account for the total number of observations. We included a bird nested within site as a random effect. We conducted an identical model selection process as that described previously, and the best model had the logit-transformed proportion of observations spent foraging as the dependent variable, the interaction of treatment and stage as the fixed effect and sex as the only covariate. To understand how the entire activity budget changed in response to simulated nectar robbing and to complement the general linear model on mean proportion of time spent foraging listed above, we also conducted a multinomial analysis. The multinomial analysis used a matrix of the count of 5 min scan-samples in which each individual was either foraging, perching, engaged in aggressive territory defence, or other (standardized for number of observations) as the dependent variables, and the interaction of treatment and stage as the predictor variable. The analysis was conducted using the “multinom” function in the package “nnet” in R (Venables and Ripley, 2002).

To analyse the effects of the robbing treatment on the diet of *A. cupripennis*, we calculated the mean proportion of unique foraging observations per individual in each of the following categories: *O. grandiflora*, insects, or anything that was not *O. grandiflora*, including insects and other plant species (Supplementary information Table S2). We then split the observations into either *O. grandiflora* or any other diet item (henceforth “non-OG”). We used LMM following the model selection process described in the previous paragraph. The best model included the logit-transformed mean proportion of plants other than *O. grandiflora* in the diet as the dependent variable, the interaction of treatment and stage as the fixed effect and bird nested within site as the random effect. As before, we also included an offset for the number of observations for each individual.

### 3. Results

We conducted nectar robbing experiments on a total of 15 individuals: 10 robbed birds and 5 control birds (Supplementary information Table S3 and Figs. S1 and S2). We obtained an average of  $175 \pm 24$  points per individual, over  $5.7 \pm 0.5$  days of tracking. During this period, birds used a mean MCP home range of  $0.073 \pm 0.012$  ha (95th kernel area =  $0.176 \pm 0.036$  ha), and moved a cumulative distance of  $1152.7 \pm 95.0$  m while being tracked.

#### 3.1. Territory size and distance flown

Tracked birds exhibited a significant response to simulated robbing in terms of minimum convex polygon (MCP), 95th kernel area, and distance flown (Fig. 2), as indicated by a significant interaction term between stage (before vs. after) and treatment (robbed vs. control) in our models (Table 1). Post-hoc tests show that this was due to significant increases in MCP, 95th kernel area, and distance flown between the before-robbing and the after-robbing categories ( $p < 0.01$ ,  $p < 0.001$ , and  $p < 0.01$  respectively; Fig. 3), indicating an expansion of the territory area. Additionally, both breeding status and treatment were significantly related to territory size, in that breeding individuals and individuals that received the robbed treatment had smaller MCP and 95th kernel area (Table 1).

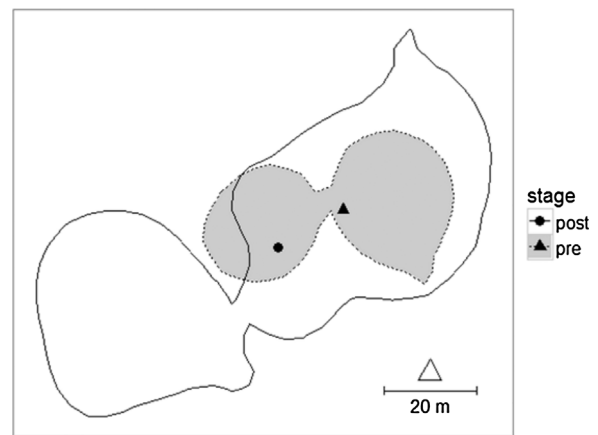


Fig. 2. Example of 95th kernel area of *A. cupripennis* territory pre- and post- nectar robbing treatment. Points indicate pre- and post- nectar robbing territory centroids.

#### 3.2. Activity budgets and diet

The diet of *A. cupripennis* before receiving the robbing treatment consisted mostly of *O. grandiflora*, with a very small portion consisting of small-bodied flying insects and nectar from other plant species (Supplementary information Table S2). The diet of *A. cupripennis* shifted to alternative, non-*Oreocallis* diet items in response to nectar robbing, as shown by the significant interaction between treatment and stage on the proportion of *O. grandiflora* in the diet (Table 2). Post-hoc tests show that this was due to an increase in other foods in the diet, namely insects or other plants, between the before-robbing and the after-robbing categories ( $p < 0.001$ ) (Fig. 4). However, there was also a significant difference between the after-control and after-robbing ( $p < 0.01$ ) categories (Fig. 4). However, there was also a significant difference between the after-control and after-robbing ( $p < 0.01$ ) categories.

The interaction of robbing treatment (robbed or control) and stage (before or after) had no significant effect on the proportion of time spent foraging (Table 2, Fig. 3). Males spent significantly more time feeding than females independent of simulated robbing (Table 2). The multinomial analysis indicated a slightly significant overall change in the number of times an individual was observed chasing (territory defence) relative to the number of times an individual was observed foraging ( $p < 0.05$ ; likelihood ratio test comparing our model against an intercept-only model), with parameter estimates suggesting that the relative number of observations in which a bird was chasing other birds increased relative to the number of observations in which a bird was foraging (Coefficient = 2.1). While not significantly different, the relative proportions of observations in which a bird was either perching or doing something else increased and decreased respectively (Coefficients = 0.40 and  $-3.68$ , respectively).

### 4. Discussion

This study is among the first to investigate how the behavioural responses of pollinators to nectar robbing may scale up to impact individual foraging ecology. We found that *A. cupripennis* hummingbirds did not abandon their territories in response to simulated nectar robbing of their main food source *O. grandiflora*, but instead expanded their territories and shifted their diet away from *O. grandiflora*, exhibiting a multifaceted response. However, *A. cupripennis* did not significantly alter their activity budgets in response to nectar robbing treatments. Overall, nectar robbing elicited changes in diet and foraging area in *A. cupripennis* that could have ramifications for its energetics as well as gene flow in the plants it pollinates.

There are several potential caveats associated with these findings. First, the number of treatment and control individuals used in this study is relatively small. However, given the challenges associated with

**Table 1**

Effect of nectar robbing vs. control treatment on *A. cupripennis* foraging ecology from general linear mixed model with treatment (robbed or control), stage (before or after), breeding status (1 = possible CP or BP, 2 = likely CP or BP, 3 = nest or copulation), sex (male or female), and an interaction term of treatment x stage as predictor variables, and individual bird identity nested within site (Ecuador or Peru) as a random effect. The fitted versions of these models by treatment are shown in Fig. 3. MCP refers to the minimum convex polygon area, 95% home range refers to the area of the 95th kernel as determined by kernel density analysis, and distance flown refers to the sum of the straight-line distances between the geographic coordinates at which an individual was observed. AIC<sub>c</sub> refers to corrected AIC values and R<sub>m</sub><sup>2</sup> refers to marginal R<sup>2</sup> values.

Effect	MCP <sup>a</sup>				95th Kernel <sup>b</sup>				Distance Flown <sup>c</sup>			
	Intercept	DF	t	P	Intercept	DF	T	P	Intercept	DF	t	P
Treat	-0.60	12	-2.02	0.07	-1.16	10	-3.73	< 0.01	-0.10	12	-0.56	NS
Stage	-0.12	11	-0.49	NS	-0.02	11	-0.14	NS	0.04	12	0.24	NS
Breed1	-	-	-	-	-0.82	10	-2.40	< 0.05	-	-	-	-
Breed2	-	-	-	-	-0.91	10	-2.56	< 0.05	-	-	-	-
Breed3	-	-	-	-	-2.27	10	-3.82	< 0.01	-	-	-	-
Sex	0.76	12	3.18	< 0.01	-	-	-	-	0.48	12	3.31	< 0.05
Treat x Stage	0.83	11	2.60	< 0.05	0.65	11	3.17	< 0.01	0.44	12	2.28	< 0.05

<sup>a</sup> AIC<sub>c</sub> = 68.22, R<sub>m</sub><sup>2</sup> = 0.47.

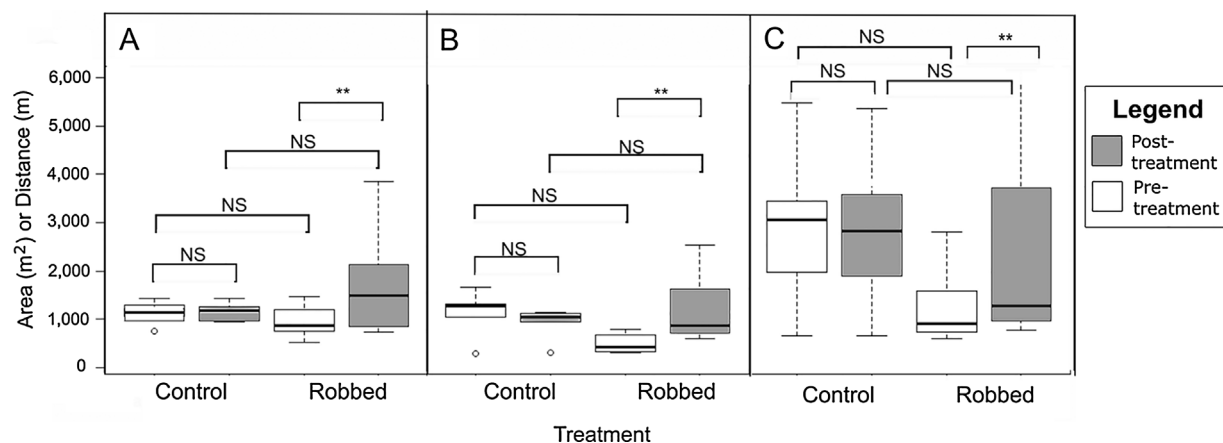
<sup>b</sup> AIC<sub>c</sub> = 66.29, R<sub>m</sub><sup>2</sup> = 0.61.

<sup>c</sup> AIC<sub>c</sub> = 37.27, R<sub>m</sub><sup>2</sup> = 0.43.

tracking tropical hummingbirds, including in some cases poorly documented natural history and the short battery life of miniature radio transmitters in extreme conditions, there are natural limitations on sample size that will be difficult to overcome until technology improves. Second, the study was conducted at two different sites, which could introduce bias in our analyses. We explicitly considered this in our statistical approach, and study site had no detectable impact on our results. Third, our post-hoc analyses showed that individuals receiving the robbed treatment had smaller before-treatment territory areas than did control individuals (Table 1). We are not aware of any a priori reason why this would influence the general findings of the study; although it is possible that individuals with larger territories may have exhibited weaker responses to the experimental treatment, exploratory one-way ANOVAs showed no significant effect of initial territory size on percent increase in MCP or 95th kernel area (results not shown). Fourth, many birds were not re-located after the initial tagging. It is possible that these birds simply had not established territories yet, or that the *A. cupripennis* populations at both sites maintain a sub-population of “floater” individuals that do not establish territories and instead forage opportunistically over larger areas (Kokko et al., 2006). We would expect the response of territory-holding *A. cupripennis* to resource reduction to be similar to our results, even if the territories are temporary (Carpenter et al., 1983). However, we do not know if the effects of nectar robbing are similar for individuals that do not hold territories. Fifth, we did not directly measure physiological condition or

energy expenditure among the individuals included in this study; doing so represents a priority for future research into the impacts of nectar robbing on pollinators. In spite of these possible limitations, our results carry great insights into the possible ramifications of nectar robbing for pollinator foraging ecology and energetics, and will be a valuable guide for future studies.

Our results suggest that nectar robbing is not costly enough to territorial pollinators to necessitate territory abandonment, as only one of the birds with an established territory that we tracked may have potentially abandoned the territory by expanding it to such an extent that successful resource defence of the entire area simultaneously was unlikely (the new foraging area included the old territory, but increased in area by 580%, see Supplementary information Table S3). Nectar robbing has a milder impact on legitimate pollinators than does the actual eradication of a resource, because in many cases robbed flowers still produce nectar. In the case of *O. grandiflora*, Hazlehurst and Karubian (2016) demonstrated that in flowers which were isolated from all floral visitors, experimental nectar robbing caused flowers to produce significantly less nectar than unrobbed flowers (6.8 μL ± 4.9 vs. 14.1 μL ± 7.9) at higher sucrose concentrations (43.3% Brix ± 11.5 vs. 30.2% Brix ± 12.4). However, there may still be a significant cost of nectar robbing to certain territorial pollinators (Irwin et al., 2010), because though robbed flowers may still produce nectar, it may be less abundant and more energetically costly to extract due to increases in viscosity associated with increased sucrose concentrations (Arizmendi



**Fig. 3.** The impacts of nectar robbing compared to control treatments on spatial foraging behavior of *A. cupripennis*. Shown are changes in (A) distance flown (m), (B) area of the minimum convex polygon (m<sup>2</sup>), and (C) area of the 95% home range (m<sup>2</sup>) within control and robbed individuals and between pre- and post-treatment (control or robbed). A white box indicates pre-treatment stage and a shaded box indicates post-treatment stage. There was no significant difference between pre- and post-control treatment individuals, and there was a significance  $P < 0.001$  between all pre- and post-robbed treatment individuals. Results of pairwise tests (motivated by significant interaction terms from a before-and-after-control mixed model) are reflected by asterisks (\* $P < 0.0125$ , \*\* $P < 0.001$ ) or by NS ( $P > 0.0125$  by Bonferroni correction).

**Table 2**

Effect tests from a before-and-after-control design general linear mixed model with treatment (robbed or control), stage (pre- or post- treatment), sex (male or female), and an interaction term of treatment x stage as predictor variables, and stage nested within individual bird identity nested within site (Ecuador or Peru) as a random effect. Activity budget indicates the mean proportion of time spent foraging, and diet represents the mean proportion of foraging observations in which the individual was not feeding on *Oreocallis grandiflora*. The fitted versions of these models by treatment are shown in Fig. 3.

Effect	Non- <i>Oreocallis</i> in diet <sup>a</sup>				Proportion of foraging observations <sup>b</sup>			
	Intercept	DF	t-value	P	Intercept	DF	t-value	P
Treatment	0.08	12	0.27	0.79	0.10	11	0.44	0.67
Stage	-0.05	13	-0.20	0.84	0.04	13	1.84	0.09
Sex	-	-	-	-	0.81	11	3.64	< 0.01
Treatment x Stage	1.19	13	4.03	< 0.001	-0.13	13	-0.75	0.47

<sup>a</sup> AIC<sub>c</sub> = 63.46, R<sup>2</sup><sub>marginal</sub> = 0.53.

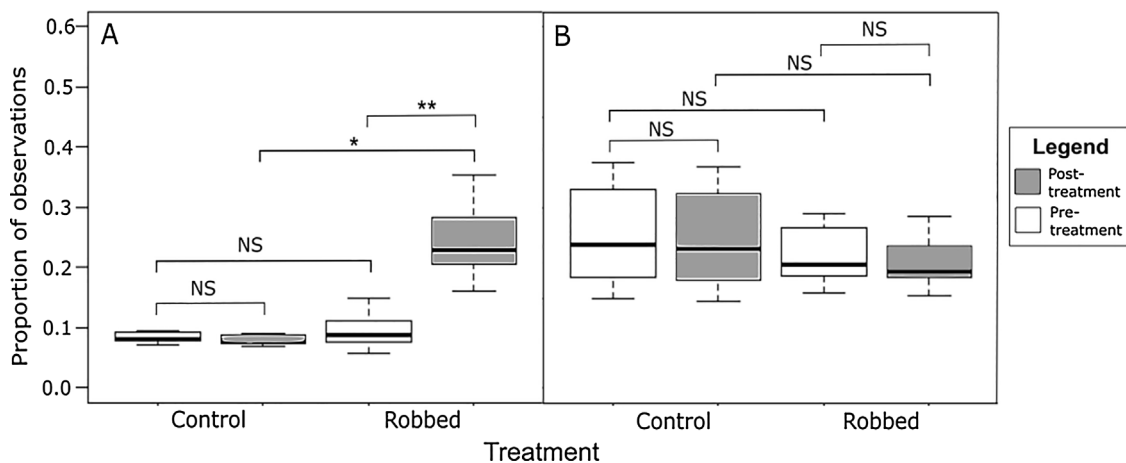
<sup>b</sup> AIC<sub>c</sub> = 53.51, R<sup>2</sup><sub>marginal</sub> = 0.41.

et al., 1996; Pleasants, 1983; Zimmerman, 1988), necessitating adaptive changes in foraging ecology. Past research has shown that pollinators including both hummingbirds and bees may avoid robbed flowers (Irwin and Brody, 1998; Maloof, 2001) and will fly farther to their next meal after visiting a robbed flower than they would otherwise (Maloof, 2001; Zimmerman and Cook, 1985). Hummingbird visitation rates to *O. grandiflora* decrease in response to nectar robbing (Hazlehurst and Karubian, 2016), suggesting avoidance of robbed flowers in this system as well. The current study confirms that, in the case of *A. cupripennis*, the effects of nectar robbing do scale up from increased inter-flower flight distance to affect territory size and overall distance flown over several days post-treatment. It is important to note that in our system we recorded a relatively low natural robbery level in *O. grandiflora* (21 ± 0.3% of flowers). However, in other systems nectar robbing has been recorded at levels as high as 100% and may vary dramatically in intensity in a single system over varying temporal scales (Irwin and Maloof, 2002).

What inferences can be made about how the territory enlargement and diet shift we observed in response to simulated nectar robbing may have impacted the energetics of individual pollinators? Nectar robbing necessitated that *A. cupripennis* fly a cumulatively greater distance, which directly increases energy expenditure, though this result was only borderline significant. The shift in diet towards presumably less desirable food sources is also likely to have impacts on energy budgets. The most common alternative food source that *A. cupripennis* turned to in response to nectar robbing were small-bodied flying insects (Supplementary information Table S2). Insect consumption has been

documented as an important source of nutrients for nectarivorous species (Gass and Montgomerie, 1981), particularly when breeding (Chavez-Ramirez and Dowd, 1992), but the digestive efficiency of insects is lower for nectarivores than other species (Roxburgh and Pinshow, 2002) and may lead to substantial weight loss in hummingbirds (Brice, 1992; Lopez-Calleja and Bozinovic, 2003). Second, it is possible, though unknown, that fly-catching is more energetically expensive than feeding on flowers in this system. Fly-catching behaviour demands rapid straight-up lift offs and extensive hovering while the birds pick insects out of the air. In comparison, while foraging on *O. grandiflora* flowers *A. cupripennis* was often able to perch while consuming nectar. Third, many of the alternative floral resources exploited by individuals in the robbed treatment were less abundant or had less nectar available than *O. grandiflora* (Supplementary information Table S5). Lastly, in a natural setting, the presence of nectar robbers such as *Diglossa* may elicit a cost in terms of territory defence for *A. cupripennis*, as every time that a *Diglossa* flower piercer entered the territory of a focal *A. cupripennis* it was chased or “dive-bombed” away, often for dozens of meters. In our before-treatment observations across all focal individuals, *Diglossa* consisted of 16.1% of all observed chases. This was less than for either conspecifics or other hummingbird species, but more than for other birds such as thrushes and owls (Supplementary information Table S4). Taken together, these findings suggest that nectar robbing exerts a significant cost on territorial pollinators, but not significant enough to necessitate territory abandonment in this system.

We observed adaptive changes in foraging ecology in response to nectar robbing in *A. cupripennis* that were consistent with resource



**Fig. 4.** Impacts of nectar robbing as compared to control treatments on foraging behavior of *A. cupripennis* in terms of (A) Changes in the proportion of observations in which non-*Oreocallis grandiflora* items were being eaten by focal hummingbirds within control and robbed treatments and between pre- and post- treatment (control or robbed). A white box indicates pre-treatment stage and a shaded box indicates post- treatment stage. There was no significant difference between before and after control treatment individuals, and there was a significant difference of  $P < 0.001$  between all pre- and post- robbed treatment individuals. Results of pairwise tests (motivated by significant interaction terms from a before-and-after-control mixed model) are reflected by asterisks (\* $P < 0.0125$ , \*\* $P < 0.001$ ) or by NS ( $P > 0.0125$ ). (B) There was no difference in mean proportion of time spent foraging in response to the experimental treatment.

reduction in other systems, including territory area expansion and diet niche expansion. In other systems, such changes in resource availability were associated with shifts in activity budgets, as in Powers and McKee (1994), wherein hummingbirds spent less time in territory defence, and in Ruby et al. (1994), wherein tortoises spent more time foraging. We incorporated measures of initial territory quality as a covariate in our model testing to control for any differences between sites and individuals that may have impacted activity budgets, but it was not a significant factor. It may be that our measurement of territory quality was not fine-scale enough to accurately estimate the kilocalories available from floral resources in each territory. While we cannot know for sure why activity budgets did not change in our study while territory area and diet did, it may in part have been because there was simply less food available, even when territory expansion was incorporated, and hence no increase in foraging time. In studies in which activity budgets did shift towards more foraging, the resources acquired by territory expansion led to no overall change in the total quantity of resources within the territory, though they were more dispersed (Gass, 1979; Gill and Wolf, 1975). Other factors apart from resources, especially population density, can impact territory size (Schoepf et al., 2015) and may have limited how much territory expansion was possible in our system, leaving birds with fewer resources within their territory than before robbing treatments, and making an increase in foraging time ineffective due to a decrease in available food. It is also possible that we did not witness a shift in activity budgets because hummingbird activity budgets may be limited by crop-emptying times (Brice, 1992), which forces them to abstain from feeding while they process the food they have already consumed.

## 5. Conclusion

Our findings speak to the importance of quantifying the plasticity of foraging behaviour in different contexts. In the case of nectar robbing, complex, mixed adaptive responses seem sufficient to allow maintenance of territorial behaviour, but robbing may have long term effects on pollinator daily energy intake, which could impact long-term pollinator demographics, distribution, and plant-pollinator mutualism stability. Better quantifying these putative physiological costs, as well as downstream consequences for pollen flow and plant reproduction, represent priorities for future work.

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## Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at <https://doi.org/10.1016/j.beproc.2018.01.001>.

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