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RESEARCH ARTICLE

Factors driving the within-plant patterns of resource exploitation in a herbivore

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Abstract

1. Selective pressures exerted on the feeding behaviour of animals have been extensively studied to understand their foraging patterns. In herbivores, specific within-plant patterns of resource exploitation have been reported, but their determinants remain poorly understood.
2. Here, we describe and decipher the determinants of the foraging pattern of the pollen beetle *Brassicogethes aeneus*, a pollinivorous insect that is a pest of oilseed rape *Brassica napus*. This insect feeds from flowers for almost all of its life cycle, except for a couple of weeks preceding blossoming. During this period, only flower buds are available and the insect destroys them to feed from the pollen they contain, causing serious yield losses.
3. We found that during this critical period, pollen beetles exhibit a stereotypic intra-inflorescence feeding pattern that depends on flower bud maturity. To explain this pattern, we first deciphered the selective pressures driving pollen beetles' feeding behaviour. Using a set of manipulative laboratory experiments, including behavioural experiments on plant tissues and artificial substrates, chemical characterization of plant tissues and performance experiments, we show that the pollen beetles' feeding behaviour does not seem to be driven by specialized metabolites or an attempt to reach an optimal nutrient balance, but rather by a process of maximization of total macronutrient intake. Next, using optimal diet choice models, we found that one aspect of the intra-inflorescence feeding pattern, the preference for young over old flower buds, could be well-explained through the lens of total macronutrient intake maximization per unit of time to access the resource.
4. Our study provides new insights into small-scale foraging patterns and highlights the need to characterize and assess the relative influence of several components of diet quality when deciphering selective pressures driving foraging patterns.

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KEYWORDS
evolutionary forces, feeding behaviour, macronutrient intake, resource accessibility, specialized metabolites

1 | INTRODUCTION

Animals living in complex and dynamic environments have evolved sophisticate foraging strategies that optimize food searching and processing to maximize growth, reproduction and survival (Pyke, 2019; Simpson et al., 2015; Simpson & Raubenheimer, 2012). Such optimization consists in limiting energy loss associated with locating and handling the food source, managing defence compounds contained in the food and increasing energy intake while insuring an appropriate balance of nutrients (Schoener, 1971; Westoby, 1978; Raubenheimer & Simpson, 2018; Pyke, 2019). In this adaptive framework, it appears that both factors directly related to the food (e.g. availability, accessibility, nutritional quality and defence compounds) or to the forager (e.g. physiological status, interactions with antagonists) can influence foraging decisions (Bee et al., 2011; Ho et al., 2019; Kraus et al., 2019; Mason et al., 2019; Vanderplanck et al., 2020; Woodard et al., 2019). However, we currently lack studies embracing several of these factors to assess their relative effect on foraging behaviours.

Insects have been widely studied to understand food exploitation patterns in herbivorous animals. Research has mostly focused on how herbivorous insects mix different plant species to reach optimal diets. However, availability, accessibility as well as defence compounds and nutritional quality of food sources can also vary within species and even within a given plant. Indeed, plant quality can depend on the organ, its degree of maturity or a particular location in this organ (Brown et al., 2003; Kouki & Manetas, 2002; Mason et al., 2019; Rodrigues et al., 2008). Therefore, individual plants represent a mosaic of patches that may be differentially profitable for herbivores, in the same way as different plant species do (Galdino et al., 2015). It can then be hypothesized that similar foraging strategies as observed in interspecific studies may be observed at such a small scale. However, few studies have sought to understand insect foraging strategies at a small scale, and most of them only focused on the influence of one resource characteristic, mainly specialized metabolites in the framework of the Optimal Defense Theory (e.g. Shroff et al., 2008; Köhler et al., 2015; Tsunoda et al., 2017; Hunziker et al., 2021).

In this study, we describe and try to understand the determinants of the foraging pattern of the pollen beetle (*Brassicogethes aeneus* F. syn. *Meligethes aeneus*, Coleoptera: Nitidulidae), a pollinivorous insect that feeds on the pollen of plants belonging to many different families (Free & Williams, 1978; Marques & Draper, 2012; Ouvrard et al., 2016). Although adult pollen beetles are generalist feeders, they have close relationships with brassicaceous plants, especially oilseed rape (OSR; *Brassica napus* L., Brassicaceae), on which they feed and oviposit (Ekbom & Borg, 1996; Free & Williams, 1978). Adults feed from flowers for almost all of their life cycle, except for a couple of weeks preceding OSR blossoming. During this period, only flower buds are available and the insect destroys them to feed from the pollen they contain, inflicting serious yield losses (Nilsson, 1987). It is known that during this critical period for the plant, pollen beetles exhibit a stereotypic intra-inflorescence pattern of resource exploitation. Indeed, females preferentially lay eggs in buds at an intermediate developmental stage (i.e. medium size buds; Hervé et al., 2015), while younger buds (i.e. small buds) are preferentially used for feeding (Ferguson et al., 2015; Nilsson, 1994; Seimandi-Corda et al., 2021). However, the factors that influence this fine-scale foraging strategy and particularly the clear preference for young flower buds over older ones are still unknown. To decipher the selective pressures that shape the feeding behaviour of pollen beetles, we follow Hervé, Delourme, Gravot, et al. (2014) by hypothesizing that, since the period during which pollen beetles feed on open flowers (~3–6 months) is much longer than the time spent feeding on closed buds (~2 weeks), their feeding behaviour should have been shaped based on the characteristics of the flowers rather than flower buds. Therefore, we use the well-known preference for flowers as compared with flower buds (Free & Williams, 1978) to reveal these selective pressures. We next test whether these selective pressures could explain the small-scale feeding pattern observed on flower buds. We decipher the selective pressures using a set of manipulative laboratory experiments including behavioural experiments on entire plants, plant organs and artificial substrates; chemical characterization of plant organs (i.e. quantification of macronutrients and defence compounds); and performance experiments. We next combine the selective pressures identified into an optimal diet choice (ODC) model to explain the intra-inflorescence foraging pattern of the pollen beetle.

2 | MATERIALS AND METHODS

2.1 | Plants and insects

Winter OSR plants ('Express' genotype) were grown as described in Hervé, Delourme, Leclair, et al. (2014). Plants were used for experiments at the BBCH growth stages 55–57 and 60–69 (Lancashire et al., 1991), that is the 'green-yellow bud stage' and the 'flowering stage' respectively. Following Hervé et al. (2015) and Figure S1), who showed that flower buds of six OSR genotypes ranging from 2.5 to 5 mm long are preferentially used for oviposition, bud maturity classes were defined as: 'young' < 2.5 mm, 'intermediate' 2.5–5 mm and 'old' > 5 mm.

Overwintered pollen beetles *Brassicogethes aeneus*, were collected in unsprayed winter OSR crops at Le Rheu and Betton (Brittany, France) and placed in controlled conditions (16:8 L:D, 12°C), where they were fed with agar discs containing commercial organic pollen from unspecified

plants (© Celtibio; 3% agar, 12% pollen and 4 $g\,l^{-1}$ Tegosept + 1.5 $g\,l^{-1}$ propionic acid as antifungal and antibacterial agents). No ethical approval was required for the use of *B. aeneus*. Insects were starved prior to all experiments in individual Petri dishes (Ø = 35 mm) containing a moistened filter paper. Following preliminary experiments, starvation durations, number of individuals per experiment and experiment durations were defined to get sufficient feeding contrasts without reaching saturation (i.e. total consumption of the food sources). Three starvation durations were conducted: 48 hr for feeding tests on entire plants or plant organs, 72 hr for feeding tests on artificial substrates and until reaching 50% of mortality for performance experiments (ensuring that energy levels prior to the experiment were low).

2.2 | Feeding tests

2.2.1 | Tests on entire plants

To describe the feeding pattern of the pollen beetle, one individual was placed on the main inflorescence of an entire plant at the green-yellow bud stage as described in Hervé, Delourme, Leclair, et al. (2014). After 3 days, the number of buds damaged by feeding was counted. Buds damaged by feeding are recognizable as they show irregular holes of usually large sizes and located anywhere on the buds, while buds damaged by oviposition show stereotypic small holes at their basis. The length (i.e. maturity) of damaged buds was measured under a binocular microscope (0.1 mm precision). Ten replicates were performed.

Data analysis

All statistical analyses were performed with the R software v. 4.0.2 (R Core Team, 2020). Our data are available from Dryad Digital Repository https://doi.org/10.5061/dryad.866t1g1sq. The mean number of buds damaged was compared between maturity classes (young, intermediate and old buds) using a Wald test applied on a GLMM including the individual plant as random factor (distribution: Poisson, link function: log; R package LME4: Bates et al., 2015 and CAR: Fox & Weisberg, 2011). Here and in the following experiments, pairwise comparisons of estimated marginal means (EMMeans) were systematically performed using the EMMEANS package (Lenth, 2019) and *p*-values adjusted using the false discovery rate correction (Benjamini & Hochberg, 1995).

2.2.2 | Tests on plant organs

To confirm the preference for flowers over flower buds and to assess the contribution of resource accessibility and resource chemical composition to this preference, dual-choice tests were performed. One individual was placed in a Petri dish (Ø = 55 mm) for 2 hr with two different food sources, and it was then recorded whether each food source had been damaged or not. Three choice tests were conducted: one flower versus one old bud (to confirm the preference for flowers), one old bud versus six anthers just excised from one old bud (to assess the influence of resource accessibility) and six anthers just excised from one flower versus six anthers just excised from one old bud (to assess the influence of resource chemical composition). Old buds rather than young buds were chosen to decipher the factors responsible for the preference for flowers, for practical aspects and to avoid any bias related to a physical factor since anthers of old buds and flowers have the same size. About 20–27 replicates were performed per choice test.

Data analysis

For each choice test, the probability of being damaged was compared between the two food sources using a Cochran's *Q* test, which considered the replicate as pairing factor (R package RVAIDEMEMOIRE, Hervé, 2021).

2.2.3 | Tests on artificial substrates

An experimental set-up based on agar discs was designed to assess the respective and relative contributions of macronutrients (either their quantity and ratio) and defence metabolites in the preference of pollen beetles for flowers. Artificial substrates consisted of 3% agar discs (Ø = 5 mm, thickness = 2 mm) supplemented with macronutrients (casein: whey 80:20 as protein source and sucrose as carbohydrate source) and/or pure standards of defence metabolites, depending on the experiment. Macronutrient and defence metabolite concentrations used in experiments varied depending on the hypothesis tested (see Results and Table S1). In all experiments on artificial substrates, two individuals were placed in a Petri dish (Ø = 35 mm) for 3 hr, with two or three discs depending on the experiment. Insects were filmed during the experiment and their movements tracked with the Ethovision XT software v. 15 (Noldus). The feeding behaviour was estimated as the cumulative duration spent on each disc. Following preliminary observations, it was assumed that individuals were feeding when on discs and that the feeding speed was constant. Thirty replicates were performed per experiment.

Data analysis

For each experiment, the total time spent on discs was compared between the treatments using a Wald test applied on a Linear Mixed Model (LMM) that included the replicate as random factor (R packages LME4 and CAR). The response was systematically square-root transformed to ensure model fitting.

2.3 | Chemical characterization of plant organs

2.3.1 | Macronutrients

Macronutrient quantification was performed on five samples of three different plant organs: anthers from flowers, old buds and young buds. Each sample comprised organs collected from 14 to 16 plants (four organs per plant), immediately frozen into liquid nitrogen then freeze-dried. Plant organs were sampled from the same plants to ensure unbiased comparisons. Total soluble proteins (P) were extracted from 10 mg of dried powder that was agitated for 15 min at room

temperature in 1 ml of acidified phosphate buffer (0.2 M, pH = 6.8), then centrifuged at 12,000 *g* for 30 min at 4°C. Quantification was performed using the Bradford's method (Bradford, 1976) and standard solutions of bovine serum albumin as references. Total digestible carbohydrates were extracted from 10 mg of dried powder in 2 ml of phosphate buffer (0.2 M, pH = 6.5) for 20 min at 95°C. After centrifugation at 14,000 *g* for 5 min at 4°C, the supernatant was collected, the extraction steps were repeated and both supernatants were pooled. Total digestible carbohydrates (C) were quantified using the hot anthrone test (van Handel, 1967) and standard solutions of glucose as references.

Data analysis

Macronutrient composition was compared between pairs of plant organs (flower vs. old buds and old buds vs. young buds) using Welch *t* tests. Tests were performed for P, C and P + C content as well as for the P:C ratio.

2.3.2 | Defence metabolites

Compounds were chosen according to previous studies (Hervé, Delourme, Gravot, et al. (2014); Hervé, Delourme, Gravot, et al. (2014); Seimandi-Corda et al., 2019): *S*-methylcysteine sulfoxide (SMCSO) and flavonols that were suspected to be phagodeterrent for the pollen beetle (Hervé, Delourme, Gravot, et al., 2014) as well as glucosinolates that are known defence compounds of Brassicaceae (Hopkins et al., 2009) although their effects on the pollen beetle was not clear (Hervé, Delourme, Gravot, et al. (2014); Hervé, Delourme, Gravot, et al. (2014)). Material (i.e. anthers from flowers and old buds) was collected as described for macronutrients, and metabolites were extracted and quantified as described in Hervé, Delourme, Leclair, et al. (2014) and Seimandi-Corda et al. (2019).

Data analysis

Metabolic profiles of plant organs were compared multivariately using a redundancy analysis (RDA) on centred and scaled data and an associated permutation test with 9999 permutations (R package VEGAN; Oksanen et al., 2018). Univariate Welch *t* tests with FDR-adjusted *p*-values were also performed to compare plant organs separately for each compound.

2.4 | Performance experiments

2.4.1 | Performance on plant organs

Here and in the following experiments, performance was assessed as the survival time following a defined feeding period. Survival was chosen over fecundity as performance estimator since pollen beetles produce eggs continuously as they feed (Ekbom & Ferdinand, 2003; Hervé, Delourme, Leclair, et al., 2014) and lay eggs only in flower buds. Providing them with flower buds where to lay eggs would have led to an impossibility to prevent them from feeding, which would have biased the experiments. One starved individual was placed for 24 hr in a Petri dish (Ø = 55 mm) with a non-limiting food source consisting of two flowers or two old buds. After the feeding period, individuals were placed in new Petri dishes (same size) with a moistened filter paper humidified every day, and their survival time was recorded through daily observations. About 41–43 replicates were performed per treatment.

Data analysis

The survival time was analysed using a likelihood ratio test (LRTest) applied on a survival regression (distribution: Weibull, link function: log), which included the treatment as explanatory variable [R packages SURVIVAL (Therneau & Grambsch, 2000) and CAR].

2.4.2 | Performance on artificial diets

Twenty-eight artificial diets differing in their P + C content and P:C ratio were designed, consisting of all combinations of four total macronutrient concentrations (P + C 45, 90, 180 and 270 g l^{-1}) and seven macronutrient ratios (P:C 1:0.5, 1:1, 1:2, 1:3.5, 1:5, 1:8 and 1:15). All diets were presented to insects in 3% agar discs (Ø = 5 mm, thickness = 2 mm). All discs included casein:whey 80:20 as P source and sucrose as C source as well as a constant amount of additional nutritional resources (i.e. dry yeasts and vitamins) and antimicrobial agents (i.e. Tegosept and propionic acid; see Appendix S1). The P and C content of dry yeasts was included in the calculations following Lihoreau et al. (2016). After starvation, one individual was offered two identical discs for 48 hr in a Petri dish (Ø = 35 mm) that also contained a moistened filter paper. After the feeding period, discs were removed and performance was estimated as above. Fifteen replicates were performed per diet.

Data analysis

The survival time was analysed using a LRTest applied on a survival regression (distribution: Weibull, link function: log; R packages SURVIVAL and CAR). The model included the total P + C concentration, the P:C ratio and their interaction as explanatory variables.

2.5 | Optimal diet choice modelling

To determine whether pollen beetles' feeding pattern at the inflorescence scale could be explained by a maximization of the total nutrient intake per unit of time, a model of ODC was used. As other optimal foraging models, ODC models are based on the profitability of each food source, which is dependent on the amount of nutrients acquired from the food sources and the handling time needed to locate, capture, manipulate and ingest these food sources. Since pollen beetles can be considered encountering both young and old buds simultaneously on a given inflorescence, the ODC model of Waddington and Holden (1979) was used. This model allows predicting the optimal proportion of each food source to be included in the diet, which maximizes total macronutrient intake per unit of time:

$$p_y = \frac{Q^2.D_y}{Q^2.D_y + \left(\frac{1+H_y}{1+H_o}\right)^2.(1-D_y)}$$

where p_y is the predicted proportion of young buds to be included in the diet, $Q = M.C$ is the ratio of total nutrient amounts ingested per bud (M: ratio of bud masses ingested, C: ratio of total nutrient concentrations per mass unit); H_y and H_o are handling times of young and old buds respectively; and D_y and $1-D_y$ are the relative densities of young buds and old buds on an inflorescence respectively. The distributions of the model parameters were estimated from a series of dedicated experiments described below and summarized in Table 1. The values of p_y were computed through simulations since most of the model parameters, that is M, C and H, were random variables. For that purpose, the central tendency and the distribution of each parameter were computed at the mean point of the two bud maturity classes, that is 1.75 mm long for young buds and 6 mm long for old buds (Table 1). The values of p_y were calculated for all values of D_y ranging from 0 to 100%, by steps of 1%. For each value of D_y, 10,000 simulations were performed in each of which parameter values were randomly sampled from their theoretical distributions. These 10,000 values allowed estimating a central tendency of p_y (considered as the median value) and an associated 95% credibility interval. Predicted proportions of young buds to be used could then be compared with real values observed in the feeding experiment on entire plants. The real relative density of young buds at the green-yellow bud stage was estimated by measuring all buds of the main inflorescence of 15 intact plants and calculating the proportion of young buds among the total amount of young and old buds. This proportion was estimated as 0.596 using a GLM (distribution: binomial, link function: logit).

3 | RESULTS

3.1 | Pollen beetles show a stereotypic intra-inflorescence feeding pattern

Feeding tests on entire plants revealed that the mean number of flower buds attacked differed significantly according to bud maturity ($\chi^2 = 34.33$, $df = 2$, $p < 0.001$). Young buds were the most damaged [EMMean number ($\pm SE$) of buds damaged: 5.18 ± 0.92] while no significant difference was observed between intermediate and old buds (1.60 ± 0.43 and 1.13 ± 0.35 respectively; young buds vs. olds buds $p < 0.001$; young buds vs. intermediate buds $p < 0.001$; old buds vs. intermediate buds $p = 0.352$).

3.2 | What selective pressures drive pollen beetle feeding behaviour?

3.2.1 | Pollen beetles prefer to feed on flowers rather than on buds and this preference is adaptive

In dual-choice tests offering one flower and one old bud, pollen beetles exhibited a total preference for flowers ($Q = 20.00$, $df = 1$, $p < 0.001$, Figure 1a). The performance experiment conducted to assess the adaptiveness of this preference showed that pollen beetles survive for a longer time when having fed from flowers ($\chi^2 = 5.08$, $df = 1$, $p = 0.024$, Figure 1b).

3.2.2 | Pollen accessibility contributes to the preference for flowers

To assess the contribution of pollen accessibility (i.e. whether or not the perianth is present) to the pollen beetle's preference for flowers, dual-choice tests offering one old bud and anthers just dissected from one old bud were conducted. The damage probability was significantly higher on anthers (proportion [95% CI]: 0.96 [0.81–0.99]) than on whole old buds (0.11 [0.02–0.29]; $Q = 21.16$, $df = 1$, $p < 0.001$).

3.2.3 | Pollen chemical composition contributes to the preference for flowers

To assess the contribution of pollen chemical composition to the pollen beetle's preference for flowers, dual-choice tests offering anthers just dissected from one flower and anthers just dissected from one old bud were conducted. The damage probability was significantly higher on anthers from flowers (0.74 [0.54–0.89]) than on anthers from old buds (0.33 [0.16–0.54]; $Q = 4.84$, $df = 1$, $p = 0.028$).

TABLE 1 Distribution of the parameters used in the optimal diet choice model

Name	Description	Unit	Distribution
M	Ratio of bud masses ingested per bud (i.e. bud mass ingested on young buds divided by the bud mass ingested on old buds)	unitless	$\mathcal{N}(\mu = 0.45, \sigma = 0.08)$
C	Ratio of total nutrient (P + C) concentrations per mass unit (i.e. total nutrient concentration of young buds divided by the total nutrient concentration of old buds)	unitless	$\mathcal{N}(\mu = 1.15, \sigma = 0.14)$
H	Handling time	min	$\begin{cases} \text{Young buds}, \mathcal{N}(\mu = 18.96, \sigma = 2.12) \\ \text{Old buds}, \mathcal{N}(\mu = 76.21, \sigma = 3.81) \end{cases}$

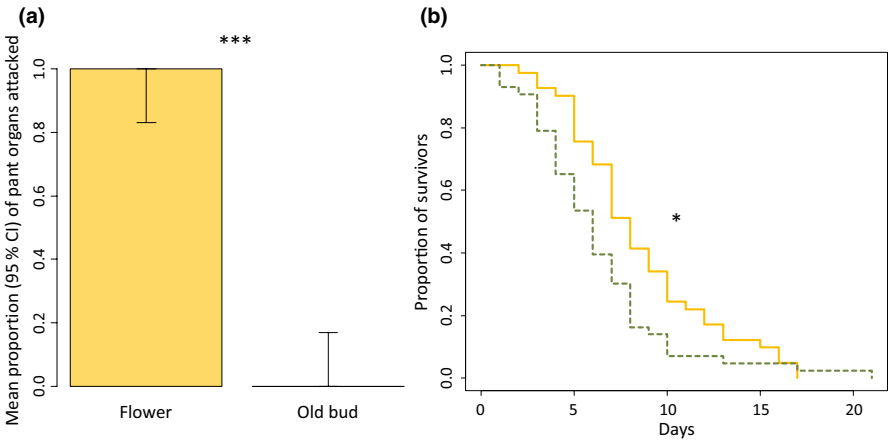

FIGURE 1 Pollen beetles prefer to feed on flowers rather than on buds and this preference is adaptive. (a) Mean proportion (95% CI) of plant organs damaged in dual-choice tests ($n = 20$). (b) Kaplan–Meier survival curves of individuals having fed on flowers (yellow) or old buds (dashed green; $n = 41–43$ per treatment). Asterisks indicate significant differences (*$p < 0.05$ and ***$p < 0.001$)

Macronutrient content differs between anthers from flowers and anthers from old buds

Quantification of the macronutrient content of anthers from flowers and of anthers from old buds revealed no difference in protein (P) concentration ($t = -0.97$, $df = 7.66$, $p = 0.363$) but revealed a significant difference in carbohydrate (C) concentration ($t = 6.60$, $df = 6.56$, $p < 0.001$), with a higher concentration in anthers from flowers (Figure 2). Consequently, organs also differed in their P:C ratio ($t = 5.32$, $df = 6.13$, $p = 0.002$) and total P + C amount ($t = 6.33$, $df = 6.05$, $p < 0.001$). The mean P:C ratio was of 1:10.2 for anthers from flowers and 1:4.9 for anthers from old buds, while the total macronutrient amount was 1.7 times higher in anthers from flowers on average (Figure 2).

Pollen beetles prefer the macronutrient content of anthers from flowers

To test whether the difference in macronutrient content between plant organs was involved in the pollen beetle's preference for flowers, dual-choice tests offering artificial substrates containing macronutrients in the same concentration and ratio as in anthers from flowers and anthers from old buds were performed. Pollen beetles spent significantly more time feeding from the diet mimicking anthers from flowers than anthers from old buds ($\chi^2 = 11.28$, $df = 1$, $p < 0.001$, Figure 3a).

Only total macronutrient content, but not their ratio, influences the pollen beetle's feeding behaviour

Since macronutrient content was proven to be involved in the pollen beetle's preference for flowers, additional experiments were performed to assess the respective influence of the total macronutrient amount (P + C) and of macronutrient ratio (P:C). Choice tests offering artificial substrates supplemented with macronutrients in the same total amount but with different ratios showed no significant preference for any diet ($\chi^2 = 2.32$, $df = 2$, $p = 0.314$, Figure 3b). On the contrary, choice tests offering artificial substrates supplemented with macronutrients in the same ratio but with different total amounts showed a significant preference for the diet containing the highest total macronutrient amount ($\chi^2 = 8.71$, $df = 1$, $p = 0.003$, Figure 3c).

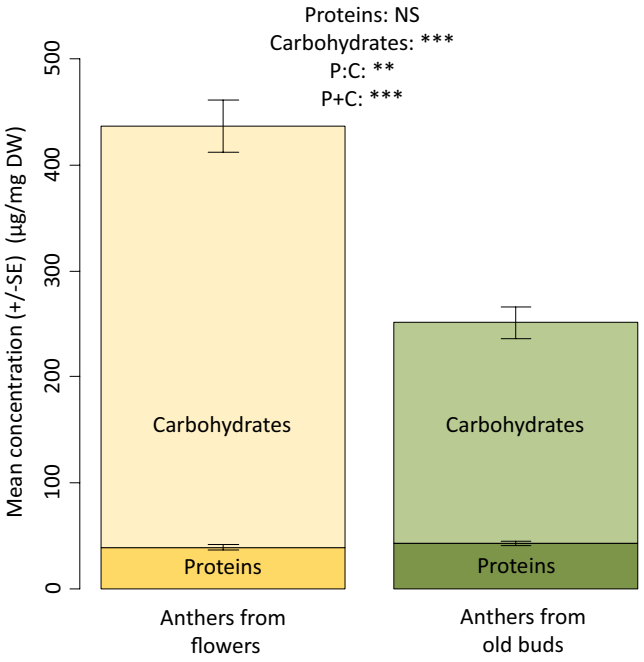

FIGURE 2 Mean concentration (±*SE*; µg/mg DW) of proteins and carbohydrates in anthers from flowers and anthers from old buds ($n = 5$ per treatment, 14–16 plants per replicate). Asterisks indicate significant differences (NS $p > 0.05$, *$p < 0.05$, **$p < 0.01$ and ***$p < 0.001$)

The maximization of total macronutrient intake shown by pollen beetles is adaptive

To assess whether the process of maximization of total macronutrient intake shown by pollen beetles is adaptive, a performance experiment with artificial diets differing in their total macronutrient amount (four treatments from 45 to 270 g/L) and ratio (seven treatments from 1:0.5 to 1:15) was conducted. No effect of the interaction between total macronutrient amount and ratio on the pollen beetles' survival ($\chi^2 = 0.03$, $df = 1$, $p = 0.872$) as well as no effect of the macronutrient ratio ($\chi^2 = 0.46$, $df = 1$, $p = 0.499$) were found. However, a significant effect of the total macronutrient amount was shown, with survival increasing with this amount ($\chi^2 = 21.53$, $df = 1$, $p < 0.001$, Figure 3d).

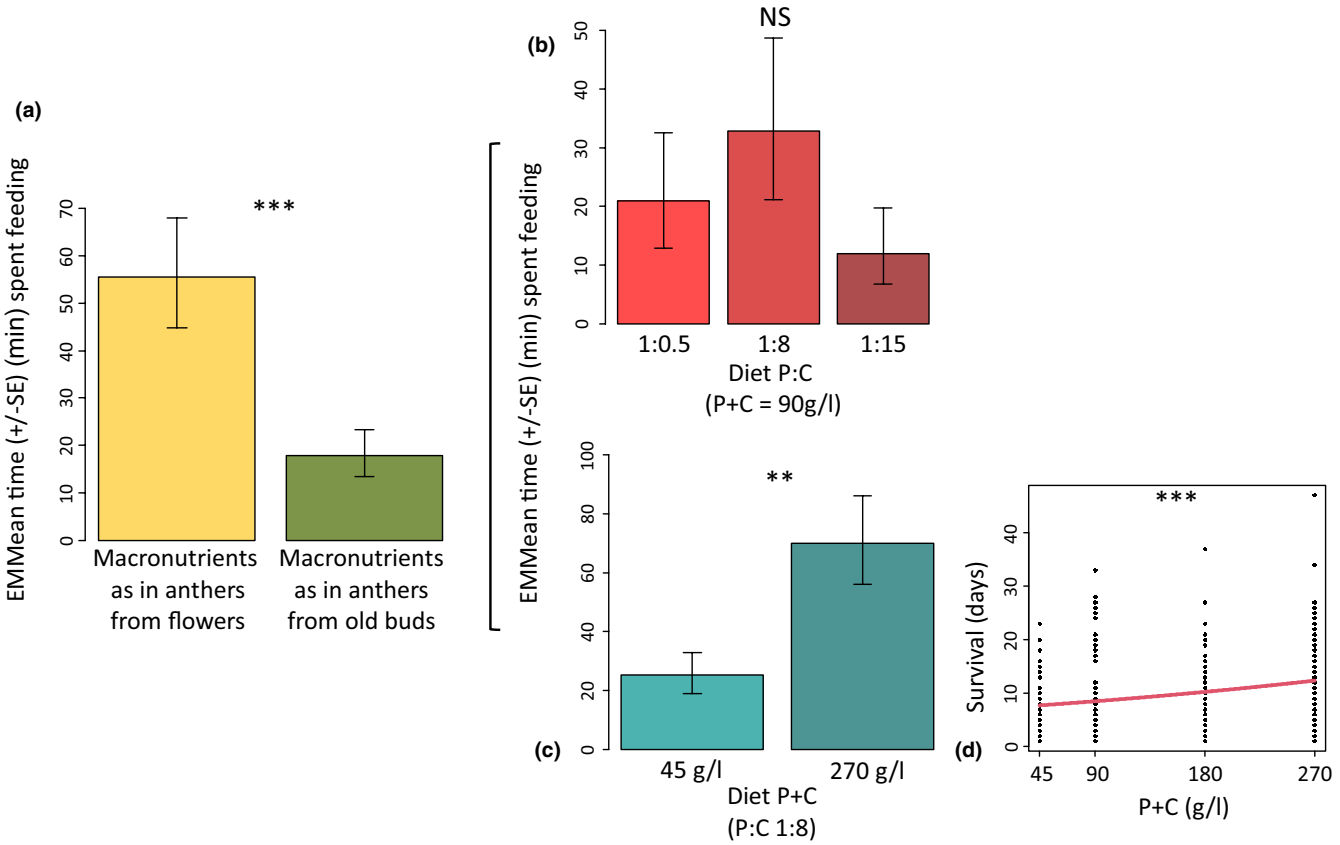

FIGURE 3 Pollen beetles maximize total macronutrient intake and this behaviour is adaptive. EMMean time (±*SE*; min) spent feeding on artificial substrates supplemented with (a) macronutrients as in anthers from flowers and anthers from old buds ($n = 30$), (b) the same macronutrient amount (P + C = 90 g/L) but with different ratios ($n = 30$) and (c) the same macronutrient ratio (P:C 1:8) but with different total amounts ($n = 30$). (d) Survival time (days) according to the total macronutrient amount. Dots represent individual points, and the red line represents the mean survival over the seven different P:C ratios. Asterisks indicate significant differences (NS $p > 0.05$, $^{*}p < 0.05$, $^{**}p < 0.01$ and $^{***}p < 0.001$)

The concentration of two glucosinolates differs significantly between anthers from flowers and anthers from old buds

To assess whether anthers from flowers and anthers from old buds differ in their content of chemical defences, three classes of specialized metabolites were quantified: glucosinolates, flavonols and SMCSO. The multivariate analysis did not reveal any difference in the global chemical profile of the two organs ($F = 0.91$, $p = 0.420$). In contrast, univariate analyses showed a significant difference in the concentration of two glucosinolates, namely progoitrin and epiprogoitrin. Both compounds were more concentrated in anthers from old buds (Table 2).

Epiprogoitrin is phagodeterrent at physiological concentration

To assess whether the difference in progoitrin and epiprogoitrin concentrations is involved in the pollen beetle's preference for flowers, dual-choice tests on artificial substrates supplemented with pure standards representing concentrations similar to those of anthers from flowers and anthers from old buds were performed. No difference in time spent feeding was found for progoitrin ($\chi^2 = 0.02$, $df = 1$, $p = 0.876$). A significant difference was found for epiprogoitrin ($\chi^2 = 4.51$, $df = 1$, $p = 0.034$), with less time spent feeding when epiprogoitrin was applied in concentrations similar to those of anthers from old buds [EMMean time ± *SE* (min) spent feeding when epiprogoitrin was applied in concentrations similar to those of flowers 21.1 ± 5.4 and in concentrations similar to those of anthers old buds 9.1 ± 2.9].

The effect of epiprogoitrin is negligible compared to the one of macronutrients

Since both the effect of the total macronutrient amount and of the concentration of epiprogoitrin were consistent with the preference of pollen beetles for anthers from flowers, two additional experiments were performed to assess the relative contribution of these two factors. First, a dual-choice test offering artificial substrates with macronutrients at the same ratio (P:C 1:8) but with total amounts as in anthers from flowers and anthers from old buds confirmed that pollen beetles spend more time feeding where total macronutrient amount is the highest (Figure 4). In the second experiment, epiprogoitrin was added to the diet to assess whether inverting the concentrations found in the two organs could cancel the effect of macronutrients. Epiprogoitrin at the concentration found in anthers from old buds was then added to the diet containing macronutrients

	Anthers from flowers	Anthers from old buds	*df*	*t*	*p*
Glucosinolates					
Epiprogoitrin	1.10 ± 0.33	3.32 ± 0.48	7.08	−3.79	**0.043**
Progoitrin	0.66 ± 0.21	2.08 ± 0.25	7.78	−4.38	**0.033**
Gluconasturtiin	0.48 ± 0.05	0.48 ± 0.05	7.77	0.00	1.000
Gluconapin	0.26 ± 0.11	1.26 ± 0.36	4.78	−2.66	0.122
Glucobrassicin	0.14 ± 0.02	0.32 ± 0.06	5.37	−2.85	0.108
Flavonols					
Flavonol 1	1.52 ± 0.10	1.30 ± 0.03	4.76	2.06	0.160
Flavonol 2	9.38 ± 0.83	8.80 ± 0.28	4.87	0.66	0.635
Flavonol 3	2.84 ± 0.15	3.34 ± 0.09	6.57	−2.79	0.108
Flavonol 4	0.00 ± 0.00	0.06 ± 0.02	4.00	−2.45	0.153
Flavonol 5	0.06 ± 0.02	0.10 ± 0.00	4.00	−1.63	0.257
Flavonol 6	0.30 ± 0.03	0.30 ± 0.04	7.20	0.00	1.000
Flavonol 7	4.70 ± 0.21	5.20 ± 0.16	7.40	1.89	0.160
S-methylcysteine sulfoxide	19.70 ± 3.73	24.00 ± 3.84	7.99	−0.80	0.578

TABLE 2 Mean concentration (±*SE*) of metabolites in anthers from flowers and anthers from old buds (nmol mg^{-1} DW), and results of Welch *t* tests (FDR adjusted *p*-values). *p*-values <0.1 are indicated in bold. *N* = 5 per bud tissue

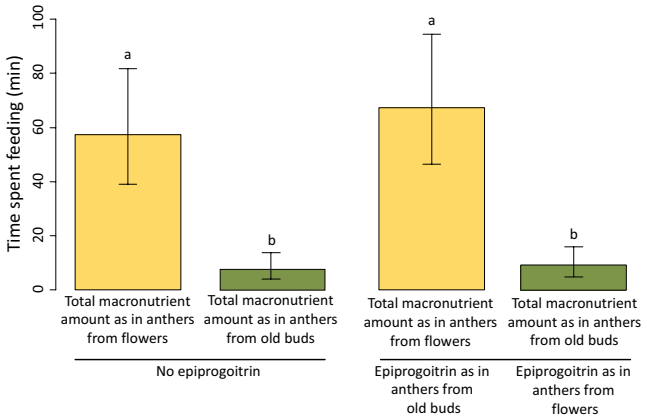

FIGURE 4 The phagodeterrent effect of epiprogoitrin is negligible compared to the effect of total macronutrient amount. EMMean time (±*SE*; min) spent feeding on artificial substrates supplemented with total macronutrient amount as in anthers from flowers and anthers from old buds, without epiprogoitrin (left, *n* = 30) or with epiprogoitrin concentrated as in the opposite organ (right, *n* = 30). Different letters indicate significant differences between the treatments

at the concentration found in anthers from flowers and vice versa. No significant change in the feeding pattern was observed (Figure 4).

3.3 | The intra-inflorescence feeding pattern of pollen beetles on flower buds is perfectly explained by an optimal foraging for total macronutrient intake per unit of time

Previous experiments showed that two selective pressures seem to drive pollen beetles' feeding behaviour: pollen accessibility and maximization of the total macronutrient intake from pollen. To assess whether the pollen beetle's intra-inflorescence foraging pattern on flower buds, that is the preference for young buds over old ones, could be explained by these two factors, an optimal foraging approach was chosen. Indeed, optimal foraging theory models the maximization of a currency (here total macronutrients) per unit of time (here handling time, i.e. time to pierce the perianth and access the pollen), which together define resource profitability. An ODC model that takes into account the relative density of both resources which are encountered simultaneously on an inflorescence was used. To parametrize this model, the mean mass ingested per bud, the total macronutrient concentration and the time to pierce the perianth per bud were estimated using dedicated experiments according to the methods described in Appendix **S2**. First, the mean mass ingested per bud mass, which was used to estimate the ratio of bud masses ingested from young vs. old buds (*M*), was more than twice as low on young buds as it was on old buds (mean mass ingested per bud ± *SE* [mg FW]: young buds 1.09 ± 0.13 and old buds 2.28 ± 0.23). Second, the total macronutrient concentration (protein plus carbohydrate), which was used to estimate the ratio of total nutrient content per unit mass of young vs. old buds (*C*) was on average 1.4 times higher in anthers from young buds than in anthers from old buds. In young buds, the mean concentrations of proteins and carbohydrates (µg/mg DW-1 ± *SE*) were 78.45 ± 4.00 and 284.52 ± 10.80 respectively; in old buds these were 42.54 ± 2.36 and 208.75 ± 14.72 respectively. Third, a strong positive relationship was found between bud maturity and handling time (Figure 5). For young (<2.5 mm) buds, the handling time (*Hy*) was *c*. 4 times shorter than for old (>5 mm) buds (*Ho*). Simulations were then performed (Appendix S2) to estimate theoretical distributions of these parameters *M*, *C* and *H* (see Table 1) to feed the ODC model. The predicted optimal proportion of young buds to be included in the diet was consistent with the observed

proportion (Figure 6), demonstrating that the intra-inflorescence feeding pattern of pollen beetles could be well-explained by a process of maximization of total macronutrient intake per unit of time.

4 | DISCUSSION

In this study, we found that an herbivorous insect, the pollen beetle, shows a stereotypic intra-inflorescence pattern of resource exploitation that depends on flower bud maturity. It has already been documented that organ maturity can drive foraging decisions in foliar herbivores (Rodrigues et al., 2008; Choong, 1996; Kouki & Manetas, 2002; Lambdon & Hassall, 2005; Blüthgen & Metzner, 2007). However, much less is known in other feeding guilds, including pollinivores. In line with field observations performed by Seimandi-Corda et al. (2021), we observed a preferential feeding on young flower buds compared to intermediate and older ones. Ekbom and Borg (1996), Hervé et al. (2015) and Seimandi-Corda et al. (2021) showed that pollen beetle females preferentially lay eggs on buds at an intermediate developmental stage. This suggests that the lower exploitation of intermediate buds for feeding might be a way to share resources that are used simultaneously for oviposition and feeding (López-Ortega et al., 2019). However, the reasons behind the clear feeding preference for young buds over old ones remained unexplained.

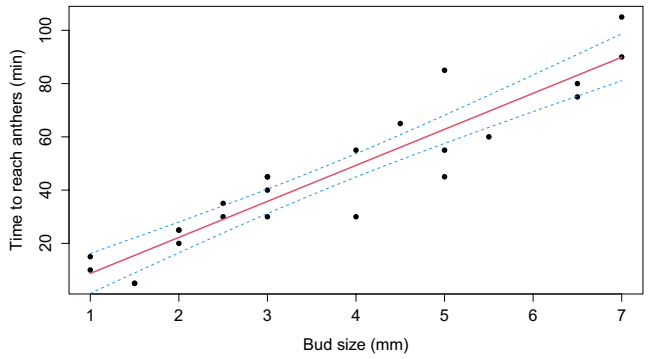

FIGURE 5 Relationship between bud maturity (mm) and handling time (min). Regression line (red) and associated 95% confidence interval (dashed blue line) are represented. $R^2 = 0.87$, $F_{1,22} = 148.2$ and $df = 1$; $p < 0.001$

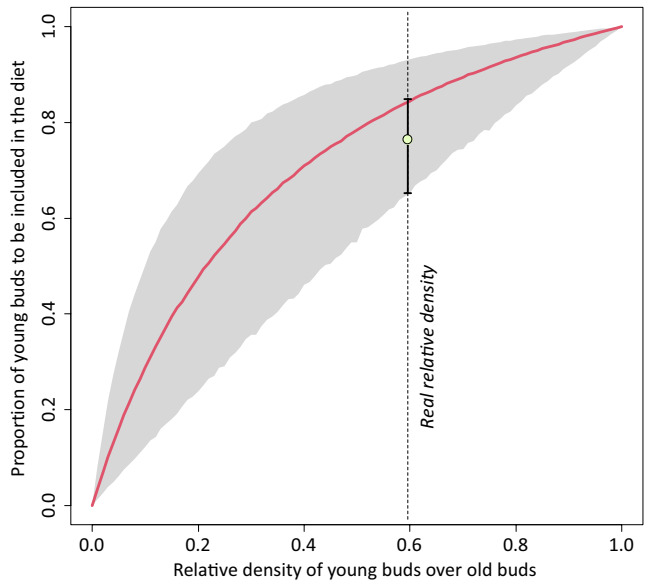

FIGURE 6 Maximization of the total macronutrient intake per unit of time explains the pollen beetle's feeding pattern on inflorescences when only flower buds are available. Proportion of young buds included in the diet according to real relative density available, as predicted by an optimal diet choice model maximizing nutrients intake per unit of time and as observed in feeding tests on entire plant. Predicted proportion: median (red line) with 95% credibility interval (grey area) from simulations. Observed value: mean (green circle) with 95% confidence interval

The most direct way to explain the feeding pattern of pollen beetles would be to identify the selective pressures driving its behaviour. In line with Free and Williams (1978), we confirmed the total preference of pollen beetles for flowers over flower buds and the adaptiveness of this preference. This supports the hypothesis proposed by Hervé, Delourme, Gravot, et al. (2014) that flower characteristics should have shaped the feeding behaviour of pollen beetles. These characteristics might be of different nature since a multiplicity of factors directly related to the food source are commonly assumed to influence foraging decisions (Stephens et al., 2007). However, a wealth of studies defined diet quality as a single currency, mainly nutrients (Jensen et al., 2012; Kohl et al., 2015; Mayntz et al., 2005; Vaudo et al., 2020) or defence content (Galdino et al., 2015; Shroff et al., 2008). So far, few studies combined the influence of several factors and most of these studies were correlative, that is they did not directly test the influence of each factor and their interaction on the forager's behaviour. Such correlative approaches might lead to a biased view of the relevant determinants of the foraging behaviour. As an example, Cronin and Hay (1996) found that although seaweed tissue toughness was correlated with the feeding behaviour of two marine invertebrates, a proper test of the influence of this factor using artificial substrates revealed that it did not explain the feeding pattern, contrary to defence compounds. Here, we characterized and assessed the contribution of resource accessibility and chemical composition, both in terms of nutrients and defence compounds, to the preference of pollen beetles.

We found that pollen beetles fed significantly less when the perianth was present. By acting as a physical and potential chemical barrier (Hervé, Delourme, Leclair, et al., 2014), the perianth can significantly reduce resource accessibility. This could partly explain the preference for flowers, where the pollen is directly accessible. Since it has been shown that structural characteristics such as tissue thickness, toughness as well as the presence and shape of trichomes, can vary according to the maturity level of plant organs (Adebooye et al., 2012; Afshari & Rahimmalek, 2018; Cronin & Hay, 1996), we hypothesized that structural characteristics of the bud perianth can vary according to the bud developmental stage and influence the feeding behaviour of pollen beetles. Indeed, we found that handling

time (i.e. time to pierce the perianth and reach anthers) was higher for old buds than for young buds. The total time required for food searching, capture and handling is a key parameter of optimal foraging models since it contributes to determining resource profitability (Barkan & Withiam, 1989). Searching time is more decisive for organisms that encounter resources sequentially, while handling time is more decisive for organisms that encounter resources simultaneously since it represents the only component of the time spent foraging. Moreover, handling time seems to depend mainly on prey mobility and size for predators (Abraços-Duarte et al., 2021; Beissinger et al., 1994), whereas resource accessibility seems of more importance for herbivores (Mallinger & Prasifka, 2017; Meire & Ervynck, 1986; Sayers & Menzel, 2012). This makes resource accessibility related to the perianth a key parameter to consider to explain pollen beetles' foraging decisions, since pollen beetles encounter all buds of an inflorescence simultaneously.

Behavioural experiments further showed the involvement of the pollen chemical composition in the preference for flowers. The impact of pollen chemistry on feeding behaviours has been reported in other pollinivores including honeybees (Cook et al., 2003). Although total energy is key for predators (Jensen et al., 2012), macronutrient quality and quantity seems of more influence for herbivores' feeding decisions (Cook et al., 2003; Vanderplanck et al., 2014; Ghosh & Jung, 2020; Felton et al., 2016; Nie et al., 2014). In this study, the quantification of macronutrient content of anthers from flowers and from old buds revealed significant differences in both the total amount and ratio of macronutrients. Feeding tests on artificial diets showed a strong preference of pollen beetles for artificial substrates incorporating macronutrients as in anthers from flowers and showed that this preference is related to the total macronutrient amount but not their ratio. Additionally, we found that this maximization of total macronutrient intake is adaptive, as it increases survival time. Our results are consistent with the nutritional heterogeneity hypothesis, which states that since generalist insect herbivores are more likely to encounter unbalanced foods, they are more prone to maximize nutrient intake to suffer less from a deficit of the limiting nutrient (Simpson et al., 2002). This strategy would reflect the greater probability that a generalist will subsequently encounter resources with a complementary imbalance (Raubenheimer & Simpson, 1999).

Apart from macronutrients, defence metabolites are regularly emphasized to explain foraging decisions of herbivores (Fraenkel, 1959; Freeland & Janzen, 1974). Here, we found that the concentration of two glucosinolates differed significantly between anthers from flowers and from old buds, with higher concentrations in anthers from old buds. Behavioural tests further showed that the difference in epiprogoitrin concentration triggered a phagodeterrent effect on the pollen beetle. A growing number of studies have reported the presence of deterrent or toxic compounds in the pollen of several plant species (Mierziak et al., 2014; Palmer-Young et al., 2019; Stevenson, 2020). It is hypothesized that the presence of defences in the pollen may serve to prevent overexploitation by pollen-feeding insects such as pollen beetles, thus ensuring pollination by beneficial species (Rivest & Forrest, 2020). Nonetheless, it has been shown that pollen defences may also deter non-specialist bees, thus affecting pollination (Arnold et al., 2014; Brochu et al., 2020). Therefore, the actual function of pollen defences and their influence on the ecology and evolution of pollinators relatively to flower characteristics (morphology, colour, scent, pollen shape and nutrient content) is still unclear (Rivest & Forrest, 2020). In this study, we disentangled the relative contributions of total macronutrient amount and epiprogoitrin concentration in the preference of pollen beetles for anthers from flowers and found that the effect of epiprogoitrin was negligible compared to the effect of macronutrients. Thus, even for a pollen antagonist such as the pollen beetle, pollen defences do not appear as a significant component of foraging decisions. This result further questions the role of plant defences in the evolution of plant–pollinator interactions.

Taken together, our results suggest that the feeding behaviour of pollen beetles is driven by both handling time, which here depends on pollen accessibility, and the total macronutrient intake from pollen. To finally test whether the pollen beetle's intra-inflorescence feeding pattern, that is the preference for young buds over old ones, could be explained by these two factors, an ODC model was used. Since pollen beetles can be considered encountering young and old buds simultaneously, we chose the model of Waddington and Holden (1979) that integrates this hypothesis. We integrated all parameters that we identified as relevant determinants of the pollen beetles' foraging behaviour (i.e. relative density, mass of buds ingested, total macronutrient concentration and handling time) in our model. Quantitative predictions from such simultaneous-encountering ODC models have typically been obtained on vertebrate predators (Cayford & Goss-Custard, 1990; Galis & de Jong, 1988; Meire & Ervynck, 1986; Sih & Petranka, 1988). Here, we predicted the proportion of young buds to be included in the diet to maximize total macronutrient intake per unit of time to access the resource by an herbivorous insect, depending on the relative density of young buds. We found that the observed proportion of young buds included in the diet was quantitatively consistent with the predicted proportion. Thus, the intra-inflorescence feeding pattern of pollen beetles, in particular the clear preference for young buds over older buds, appears to be driven by their faster handling time and their slightly higher macronutrient content, which both compensate for the lower ingested mass on this resource.

Overall, this work highlights the need to characterize and assess the influence of several components of diet quality on the foraging decisions of animals. It also shows the importance of deciphering evolutionary forces shaping foraging behaviours, although this represents significant conceptual and practical difficulties due to the number of factors involved and the need to determine the relative contribution of each in a quantitative manner.

AUTHORS' CONTRIBUTIONS

M.H., A.M.C., G.S.C. and L.B. conceived the ideas and designed the methodology; M.H., G.S.C., K.M., L.B., M.T., C.L. and J.O. collected the data; M.H., G.S.C. and L.B. analysed the data; L.B. wrote the first

draft of the manuscript and all authors contributed to revisions. All authors gave final approval for publication.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

DATA AVAILABILITY STATEMENT

Data have been deposited on Dryad (https://doi.org/10.5061/dryad.866t1g1sq).

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