Mismatched diets: defining the nutritional landscape of grasshopper communities in a variable environment

DOUGLAS LAWTON,1,† MARION LE GALL,2 CATHY WATERS,2,3 AND ARIANNE J. CEASE1,2

1School of Life Sciences, Arizona State University, Tempe, Arizona 85281 USA
2School of Sustainability, Arizona State University, Tempe, Arizona 85281 USA
3NSW Department of Primary Industries, Dubbo, New South Wales 2000 Australia

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Abstract. Animal populations must be able to acquire an adequate amount of nutrients to persist regardless of what environment they are in. In highly variable environments, such as drylands where food sources are limited, this potential mismatch between physiological demands and what is available in the environment is accentuated. For herbivores, the balance of macronutrients (protein and carbohydrate) is particularly important and both nutrients are highly variable in plants both spatially and temporally. Whereas it is known that many herbivores will forage multiple plants to achieve an optimal nutritional ratio (termed the intake target), it is less known how herbivores with different life history strategies address this in variable environments. In this study, we measured the intake targets of three grasshopper species with differing life history strategies, two migratory and one non-migratory, at three locations in New South Wales, Australia. We measured nutrient variation in plants spatially and temporally by sampling three different locations and repeated the measurement twice for one of these locations. At all three locations and both times, host plant protein differed substantially but carbohydrate content remained constant. The non-migratory grasshopper species shifted their intake target, presumably to redress nutrient imbalances. On the other hand, the two migratory grasshopper species largely maintained the same intake target, even when in a nutritionally suboptimal environment. These results suggest that non-migratory species are likely more limited in their capacity to forage for optimal diets and may rely more on digestion to survive in nutritionally suboptimal locations. In contrast, migratory grasshoppers may migrate to obtain the nutrients they need instead of redressing imbalances locally. Therefore, a strong metapopulation structure may aid in the persistence of migratory species at larger spatial scales. Since herbivores, especially insects, are important from nutrient cycling to food chains, understanding how populations persist in nutritionally variable environments is important to the overall ecosystem functioning. Further research should consider how nutritional demands drive population dynamics and how it changes with life history strategies.

Key words: Acrididae; Australia; drylands; grasshoppers; insect herbivory; nutritional ecology.

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† E-mail: ddlawton@asu.edu

INTRODUCTION

Environmental variability has an important influence on animal population dynamics (Bjornstad and Grenfell 2001) especially in drylands (Noy-Meir 1973, 1974, Whitford 2002). For example, female kangaroos (Macropus spp.) can delay egg development for months until food is available (Clark and Poole 1967, Dawson 1995). This creates boom and bust population dynamics that
correspond with the variable rainfall of inland Australia (Caughley et al. 1985, Morton et al. 2011). Nutrient availability is generally recognized as an important driver for animal populations (Andrewartha and Birch 1954, White 1993, Denno 2012, Hunter et al. 2012). However, few studies have looked at the relationship between free-ranging herbivore nutritional demands and available plant nutrient dynamics within variable environments (Nie et al. 2015). In this study, we examined how the nutritional preferences of three grasshopper species, two migratory and one non-migratory, vary with grassland nutrient content.

Plant nutrient contents (e.g., macronutrients like protein and carbohydrate or elements like carbon, nitrogen, and phosphorus) are more variable than the body composition of herbivores (Elser et al. 2000). To address this imbalance, generalist herbivores graze multiple plants to acquire an optimal nutrient blend, termed the intake target (Simpson and Raubenheimer 1993, 2012, Behmer 2009). The balance of protein and carbohydrates is particularly important for insect herbivores (Behmer 2009). For example, Mongolian locusts (Oedaleus asiaticus) restricted to suboptimal diets that were either too high in protein or carbohydrates had reduced survival, growth rate, and migratory capacity compared to individuals given an optimal diet (Cease et al. 2012, 2017). When confronted with suboptimal nutritional landscapes, herbivores can behaviorally select for optimal food sources (pre-ingestively regulate), ingest suboptimal foods and extract optimal nutrients in the gut (post-ingestively regulate), or a combination of both (Behmer 2009). One option to pre-ingestively regulate nutrients may be migration. Migration is an important aspect for many animals ranging from vertebrates to invertebrates (Dingle 2014), and with global change, migrations are being altered or disappearing completely (Wilcove and Wikelski 2008). Understanding the nutritional foraging strategies of migratory animals can illuminate mechanisms of their persistence in variable environments.

Grasshoppers (Orthoptera: Acrididae) are the dominant herbivores in grasslands (Branson et al. 2006) and are important for nutrient cycling (Belovsky and Slade 2000), food chains (Gandar 1982, Schmitz 1994), and can threaten food security as pests (Cease et al. 2015, Cullen et al. 2017). Therefore, understanding how grasshoppers meet their nutritional demands in the face of environmental variability has important multifaceted implications from conservation to agriculture. Migration is a common trait in grasshoppers, especially in locusts. For example, the Australian plague locust (Chortoicetes terminifera, Walker 1875) is able to fly over 500 km in a single night (Symmons and McCulloch 1980). However, there is a spectrum of migratory capacity within grasshoppers with some species entirely lacking flight capacity (e.g., the Plains lubber grasshopper, Brachystola magna) to extreme migratory capacity (e.g., locusts). This difference likely impacts how individuals meet nutritional demands with some species being able to access more plants (e.g., migratory species) than others (e.g., non-migratory species).

In this study, we investigated the spatiotemporal variation in grass nutrient contents and intake targets of three grasshopper species: C. terminifera, Oedaleus australis (Saussure, 1888), and Aiolopus thalassinus dubius (Willems, 1923) at three locations in New South Wales, Australia. C. terminifera and O. australis are highly migratory (Rentz 2003) while A. thalassinus is likely non-migratory (Haefez and Ibrahim 1962, Heifetz and Applebaum 1995; D. Rentz, personal communication). Since grass nutritional content changes with phenological stages, we predicted that the nutritional landscape for grasshoppers would temporally change, with early season grasses (e.g., sprouting/growing) being higher in protein (Skarpe and Bergström 1986). Spatially, since the marginal croplands of eastern Australia have a higher soil fertility than the arid interior (Morton et al. 2011), we predicted that grasses closer to the coast would have more protein relative to those further inland (Fig. 1). Since migratory species can migrate to locate nutritionally optimal plant sources, we expect them to keep their intake targets constant. Whereas non-migratory species have access to fewer plants, we expect them to change their intake targets to reflect a given environment, as we predict they will rely more heavily on post-ingestive regulation.

**Methods**

**Natural history of species**

*Chortoicetes terminifera* is a widely distributed grasshopper throughout Australia’s arid interior
(Hunter et al. 2001, Chapuis et al. 2011) and can migrate long distances given optimal weather patterns (Symmons and McCulloch 1980). This species is also a major economic pest of rangeland crops (Adriaansen et al. 2015). Whereas other species in the genus are pests (Le Gall et al. 2019a), *O. australis* is not a pest species but is widely distributed and highly migratory (Rentz 2003). *A. thalassinus* is not a pest species; however, little has been reported on this Australian subspecies (Rentz 2003). All species are likely grass generalists, although more is known about *C. terminifera* than either *O. australis* or *A. thalassinus* (Key 1945, 1959, Bernays and Chapman 1973, Rentz 2003).

**Field surveys**

Field selection.—We selected three locations where we were likely to find significant grasshopper populations throughout New South Wales, Australia: Trangie, Coonamble, and Ivanhoe (Fig. 1). At each location, two fields (~5–10 km²) were selected to account for localized variation. Due to a low regional population of grasshoppers, we were only able to collect data from one field in Ivanhoe. All fields except for Ivanhoe were used for livestock grazing and dominated by native grasses. The Ivanhoe field was a grassy roadside area. On the landscape level, the areas surrounding Trangie and Coonamble included a mixture of crop fields (predominately wheat) and grazing areas, whereas Ivanhoe was predominantly made of grazing fields (Jackson et al. 2018).

Vegetation survey.—To define the nutritional landscape, we conducted vegetation surveys using the relevé method (Poore 1955, Minnesota Department of Natural Resources 2013) and collected plant samples for nutrient analyses. In general, assessing plant communities is difficult; however, the relevé is a common technique that balances efficiency and time and is widely used (Mueller-Dombois and Ellenberg 1974, Barbour et al. 1980, Moore 1986, Committee 2000). We randomly selected 5 points within each field. Each point was at least 10 m from the field edge and 20 m from each other. The sampling quadrat was 5 × 5 m. Within the quadrat, we recorded total aboveground vegetation cover, percent manure, litter, rock, and the top three plant species cover (Appendix S1: Tables S1, S2, Fig. S1). All cover variables were given an ordinal
categorical level as follows: 0 = 0% cover, 1 = 1–5% cover, 2 = 6–10% cover, 3 = 11–25% cover, 4 = 26–50% cover, 5 = 51–75% cover, and 6 = 76–100% cover as done previously (Minnesota Department of Natural Resources 2013, Word et al. 2019).

For nutrient analysis, we only collected leaves and not stems since they are preferentially eaten by grasshoppers and avoided dead or dry plant material. Leaves of each species were collected randomly from individual plants throughout the quadrant and gathered in one species-specific sample. Plants were put into a drying oven at 60°C for 48 h soon after sampling. Dried samples were carried back to Arizona State University and ground using a Retsch MM 400 ball mill for 30 s at 200 rpm. To measure leaf carbohydrate and protein content, we used the Bradford protein (Bradford 1976) and phenol-sulfuric carbohydrate (Dubois et al. 1956) colorimetric assays following the Deans et al. (2018) procedure.

**Grasshopper abundance.**—To determine how grasshopper populations changed, we sampled grasshoppers at each field site and time point. The collection dates were as follows: Trangie early, 19 October 2017; Trangie late, 23 and 24 November 2017; Coonamble, 29 November 2017; and Ivanhoe, 3 December 2017. We also recorded temperature, geographical coordinates, and wind speed (Ambient weather WM-4; Appendix S1: Table S3). Grasshopper abundance was assessed with sweep netting along 5 100-m transects evenly distributed throughout the field. At the end of each transect, sweep net content was stored in a gallon plastic bag and placed in a cooler on ice. To avoid sampling bias, the same observer (D. Lawton) recorded all grasshopper abundance data. Bags were then frozen and stored at −20°C for two days after which grasshoppers were identified to species level if possible and counted as either adult or nymph (Appendix S1: Table S4). For a given time point (early or late in the season) and location, we collected all data on the same day within 6 h.

**Grasshopper intake target experiment**

**Grasshopper collection.**—We attempted to collect 24 adult individuals (12 males and 12 females) for each species at each location and time point where they were present. However, due to the population sizes we were unable to collect 24 of each species and there were significant die offs in some populations (Appendix S1: Table S5). We performed intake targets on adult grasshoppers only because they were usually the most dominant life stage and more accessible overall (Appendix S1: Table S5). Grasshoppers were collected in the same field, kept in mesh cages for a maximum of 24 h, and fed a mix of grass species from the area they were collected until they were added to the intake target experiment.

**Diet preparation.**—To control for nutrient availability within the experiments, we used isocaloric artificial diets made up of 42% macronutrients (differing ratios of protein and carbohydrates), 32% cellulose, and 4% of salt, sterols, and vitamins. All diets were made in the laboratory at ASU and kept in a freezer whenever possible following protocol similar to other studies (Clissold et al. 2014, Cease et al. 2017, Le Gall et al. 2019b) which was developed by Simpson and Abisgold (1985) from Dadd (1961).

**Intake target collection.**—To confirm that populations were regulating protein and carbohydrates, individuals from each species received two synthetic diet pairings differing in their protein (P) and carbohydrates (C) ratios (P35:C7 and P7:C35 or P28:C14 and P7:C35; Chambers et al. 1995). Grasshoppers were kept in plastic containers (26 × 20 × 5 cm) with holes for aeration for three days. Each cage contained one pair of diet dishes, a water tube, and a perch. For each cage, we collected grasshopper initial and final mass and consumption.

We ran the experiments at the Trangie Agricultural Research Centre, Trangie, NSW where grasshoppers were exposed to the natural photoperiod (12:12 h L:D). In addition, we hung 6 60-W light bulbs above the cages and set an automatic timer to turn on between 10:00 and 16:00. We recorded temperature (°C) and relative humidity (RH) using Hobos (Thermochron, Maxim Integrated) placed on the shelves in between cages (Appendix S1: Table S6). All diets were kept in a freezer until needed. Afterward, we dried all diet dishes for 48 h at 60°C which allowed us to control for biases from water absorption.

**Spatial change in intake target**

At each location, we ran intake target experiments on the two or three dominant grasshopper...
species: (1) Trangie, *C. terminifera* and *A. thalassinus* (started on 11 November 2017); (2) Coonamble, *C. terminifera* and *O. australis* (started on 29 November 2017); and (3) Ivanhoe, *C. terminifera*, *A. thalassinus*, and *O. australis* (started on 4 December 2017).

**Temporal change in intake target**

We repeated the experiment twice for *C. terminifera* grasshoppers caught at the Trangie field station. The early intake target was collected on 13 October 2017 and late intake targets on 11 November 2017.

**Statistical approach**

All data were assessed for normality and heteroskedasticity before statistical analyses. Transformations or nonparametric analyses were conducted where appropriate. For grass nutrients, we performed ANOVAs, Tukey HSDs for spatial variation and Welch’s *t* tests for temporal variation tests (e.g., Fig. 2). Traditionally, intake targets are analyzed through multivariate analysis of covariance (MANCOVAs) with initial starting mass as a covariate (Le Gall et al. 2019b). However, we used generalized additive models because they could be constructed with the same statistical family (multivariate Gaussian distribution; Wood 2017) and have the added benefit of selecting for nonlinear relationships. This allowed us to statistically test whether there was a trend (linear or nonlinear) between macronutrient content consumption and initial body mass. We constructed a model for each species with the dependent variables being total protein and carbohydrates consumed and independent variables being location, sex, treatment pair (to determine non-random feeding), and initial body mass. To determine the best fit models, we first used null space parametrization (Marra and Wood 2011) then Akaike information criterion (AIC). Since we were interested in sex and treatment differences, we left these in the models and selected only for the inclusion of initial body mass being either a nonlinear effect, linear effect, or removed entirely for each macronutrient (Appendix S1: Tables S7–S9). To determine how well each intake target overlapped with the available grass nutrients, we calculated Euclidean distances of each grass sample to the intake target slope (the ratio of P:C) using the following equation, similar to Le Gall et al. (2019):

$$\frac{m(x_o) - y_o}{\sqrt{1 + m^2}}$$

where *m* is the slope of the intake target, *x_o* and *y_o* are each unique grass specimen’s coordinates. Positive Euclidean distances represent plants that are protein biased and negative distances are carbohydrate biased, relative to the grasshopper population intake target. To test for differences between intake targets and plant nutrient contents, we used one-sample *t* tests with *μ* = 0. To test the differences between species dominance within communities, we conducted MANOVAs for spatial variation and *t* tests for temporal variation tests. Since there are issues with depicting changes in nutrients as ratios (Raubenheimer 1995), we have included separate plots with intake targets represented as slopes for each species by location for comparison (Fig. 5). This allows for visualization of changes in both nutrients that may be masked by taking a simple ratio. All statistics were conducted in the *tidyverse* framework (Wickham 2018) within the R statistical environment (R Core Team 2018). Generalized additive models were constructed with mgcv (Wood 2017) and validated with mgcvViz (Fasiolo et al. 2018). Multicomponent analyses were conducted in multcomp (Hothorn et al. 2009).

**RESULTS**

**Spatial variation**

**Grass nutrient content.**—Grass protein content varied while carbohydrate content did not significantly differ among sites (Fig. 2A). Grass protein content at Trangie was significantly higher than at either Coonamble or Ivanhoe (Fig. 2A). This shift in the protein content led to a change in the overall P:C ratio from being more carbohydrate biased in Coonamble and Ivanhoe to protein biased in Trangie.

**Grasshopper abundance.**—Grasshopper species dominance varied spatially (MANOVA *F*_4,19 = 21.341, Pillai’s = 1.620, *P* < 0.001). *C. terminifera* was the dominant species in Ivanhoe, *A. thalassinus* was the dominant species in Trangie, and *O. australis* was the dominant species in Coonamble (Fig. 3A; Appendix S1: Table S10).
Intake target variation.—Both *C. terminifera* and *A. thalassinus* intake targets changed between locations, whereas *O. australis* did not (Figs. 4, 5; Tables 1, 2). However, *A. thalassinus* intake targets changed considerably more than *C. terminifera* populations. *C. terminifera* intake targets at Trangie and Ivanhoe were roughly 0.55 P:1 C and changed to 0.73 P:1 C in Coonamble while *A. thalassinus* was 1 P:1 C in Trangie and 0.58 P:1 C in Coonamble (Table 3; Appendix S1: Figs. S1, S2).

*Aiolopus thalassinus* carbohydrate consumption was significantly higher in Trangie than Ivanhoe populations (Fig. 4A; Table 1). All *C. terminifera* populations had roughly the same intake targets with the Ivanhoe population slightly changing protein consumption. The Coonamble population ate more macronutrients combined than either Trangie or Ivanhoe even when body mass was accounted for (Figs. 4B, 5A, D, G).

Intake target and nutritional landscape.—For both *C. terminifera* (*t* test, *t* = 3.583, *df* = 15, *P* = 0.003) and *A. thalassinus* (*t* test, *t* = 3.155, *df* = 15, *P* = 0.007), the grass at Trangie was more protein biased than their intake targets.
There was a general trend of grasses at Ivanhoe being too carbohydrate biased compared to their intake targets for both *A. thalassinus* and *C. terminifera* (Figs. 5A, B, 6A). *O. australis* had landscapes that matched their intake targets (Figs. 5C, H, 6A).

### Table 1. Generalized additive model parametric coefficient results for the spatial change of macronutrient consumption (carbohydrate and protein) by species.

| Macronutrient Variable | Estimate | SE  | Z   | P    |
|------------------------|----------|-----|-----|------|
| **Chortoicetes terminifera** | Intercept | 0.12 | 0.011 | 10.901 | ≤ 0.001 |
| Carbohydrates | Ivanhoe | −0.047 | 0.009 | −5.328 | ≤ 0.001 |
| | Trangie | −0.047 | 0.009 | −5.078 | ≤ 0.001 |
| | Sex | −0.06 | 0.017 | −3.6 | ≤ 0.001 |
| | Treatment | 0.005 | 0.006 | 0.733 | 0.464 |
| **Protein** | Intercept | 0.069 | 0.015 | 4.657 | ≤ 0.001 |
| | Ivanhoe | −0.028 | 0.014 | −1.994 | 0.046 |
| | Trangie | −0.039 | 0.014 | −2.734 | 0.006 |
| | Sex | −0.01 | 0.012 | −0.863 | 0.388 |
| | Treatment | −0.009 | 0.01 | −0.889 | 0.374 |
| **Aiolopus thalassinus** | Intercept | 0.023 | 0.009 | 2.672 | 0.008 |
| Carbohydrates | Ivanhoe | 0.033 | 0.008 | 4.222 | ≤ 0.001 |
| | Trangie | −0.006 | 0.013 | −0.447 | 0.655 |
| | Sex | −0.005 | 0.007 | −0.834 | 0.404 |
| | Treatment | 0.006 | 0.006 | 0.951 | 0.341 |
| **Protein** | Intercept | 0.018 | 0.006 | 2.899 | 0.004 |
| | Ivanhoe | 0.012 | 0.006 | 1.85 | 0.064 |
| | Trangie | −0.005 | 0.007 | −0.834 | 0.404 |
| | Sex | −0.005 | 0.006 | −0.889 | 0.374 |

**Note:** SE, standard error. Models were selected via AIC and can be seen in Appendix S1: Tables S7–S9. Bold values indicate significance.

### Table 2. Generalized additive model nonparametric coefficient results for the spatial change of macronutrient consumption (carbohydrate and protein) by species. *Oedaleus australis*’ model did not have nonparametric coefficients.

| Species | Macronutrient Variable | edf | Ref.df | X²  | P    |
|---------|------------------------|-----|--------|-----|------|
| **Chortoicetes terminifera** | Carbohydrates | Grasshopper weight | 1.565 | 9 | 11.461 | ≤ 0.001 |
| **Aiolopus thalassinus** | Carbohydrates | Grasshopper weight | 2.231 | 9 | 19.85 | ≤ 0.001 |

**Note:** Models were selected via AIC and can be seen in Appendix S1: Tables S7–S9. Edf is estimated degrees freedom. Bold values indicate significance.

(Figs. 5D, E, 6A). There was a general trend of grasses at Ivanhoe being too carbohydrate biased compared to their intake targets for both *A. thalassinus* and *C. terminifera* (Figs. 5A, B, 6A). Between locations, there were mismatches of plant nutrients and intake targets for...
C. terminifera and A. thalassinus, but not O. australis. For the different C. terminifera populations, grass nutrients differed from their intake targets (ANOVA $F_{2,36} = 8.671, P = 0.001$), with Trangie being more protein biased than Coonamble and Ivanhoe (Tukey HSD, Trangie-Coonamble $P = 0.041$; Trangie-Ivanhoe $P = 0.004$; Figs. 6A, 7A). For A. thalassinus populations, grass nutrients differed from their intake targets ($t$ test, $t = 3.555, df = 10.304, P = 0.005$), with Trangie being protein biased and Coonamble being more carbohydrate biased (Figs. 6A, 7B). For O. australis, grass nutrients did not differ from their intake targets between locations (Figs. 6A, 7D).

**Temporal variation**

Vegetation.—We found a similar trend for temporal variation as spatial variation in that protein differed (Welch two-sample $t$ test, $t = 6.270, df = 41.60, P < 0.001$) and carbohydrates remained constant (Welch two-sample $t$ test, $t = -1.650, df = 48.6, P = 0.105$) between early and late season. Protein content was higher early in the season as compared to late (Fig. 2B). This led to a change in the overall P:C ratio from protein biased in the early season to carbohydrate biased four weeks later.

**Grasshopper abundance**

C. terminifera had low abundances in Trangie early and late in the season (Fig. 3B). There was no significant difference between early and late season C. terminifera or other grasshopper species dominance (MANOVA, Pillai’s $0.116, F_{2,17} = 1.13, P = 0.351$).

Intake target variation.—Temporally, C. terminifera intake targets did not significantly differ (Table 4).

Intake targets and nutritional landscape.—The available nutritional landscape was more protein biased than the intake targets in both early ($t$ test, $t = 12.790, df = 14, P ≪ 0.001$) and late ($t$ test, $t = 10.670, df = 14, P ≪ 0.001$) season for C. terminifera (Fig. 6B). Between the two time points, early season was more protein biased than late season as compared to C. terminifera’s intake target (Figs. 5F, I, 6B).

**DISCUSSION**

In this study, we found that at both spatial and temporal scales, host plant protein content significantly differed whereas carbohydrate content remained constant (Fig. 2). This was expected as early season plants had higher protein content than later season plants, and the site furthest east in the marginal croplands (Trangie) had higher protein in pastures than sites closer to the arid interior (Figs. 1, 2). Despite the spatiotemporal shift in plant nutrient contents, both migratory grasshopper species collected from across these environments largely maintained the same preferred ratio of protein to carbohydrates when given a choice. One species (C. terminifera) kept the ratio constant even in suboptimal locations. In contrast, the non-migratory species changed its intake target greatly across environments, potentially to redress a nutrient imbalance driven by the locally available plants.

To persist in variable environments like the Australian arid interior, animals need to be flexible foragers, obtaining the nutrients required to meet environmentally driven and phenological demands. For example, animals will select diets that help mediate trade-offs between migration (Rankin and Burchsted 1992), reproduction (Joern and Behmer 1997), and growth (Joern and Behmer 1997, Raubenheimer and Simpson 2003). Some grasshoppers may be able to better post-ingestively regulate to meet these demands while

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Table 3. Tukey multiple comparison results for Chortoicetes terminifera macronutrient consumption.

| Macronutrient | Comparison    | Estimate | Error  | Z value | $P$ value |
|---------------|---------------|----------|--------|---------|-----------|
| Carbohydrate  | Ivanhoe       | -0.047   | 0.009  | -5.328  | < 0.001   |
|               | Trangie       | -0.047   | 0.009  | -5.078  | < 0.001   |
|               | Coonamble     | <0.001   | 0.008  | -0.003  | 1.000     |
| Protein       | Ivanhoe       | -0.031   | 0.014  | -2.156  | 0.078     |
|               | Trangie       | -0.043   | 0.015  | -2.917  | 0.010     |
|               | Coonamble     | -0.012   | 0.013  | -0.975  | 0.592     |

Bold values indicate significance.
others might employ migration to find adequate nutrients. Our results suggest that different herbivore foraging responses may reflect the unique life histories of migratory vs. non-migratory species in variable environments. However, our study was restricted to three species. We hope our initial findings spur more comprehensive studies in the future.

Plant nutrient content

Plant nutrients varied spatially and temporally in protein but not in carbohydrate content. A change in protein content is likely influenced by species composition, plant phenology, and climatic factors. Soil nutrient uptake varies considerably between species, and it is thought uptake differences are accentuated with nitrogen.
(Bradshaw et al. 1964, Lunt 1972). Since most plant protein is in the form of nitrogen (White 1993), a spatiotemporal variation in protein content is not unexpected. Ivanhoe is not within the cropping zone and was a road side, unlike Trangie and Coonamble which likely have a history of fertilizer application and/or greater naturalized legume populations (Jackson et al. 2018). Therefore, grasses in Ivanhoe may have less soil nitrogen available to uptake. However, Coonamble had similar protein levels as Ivanhoe which may be an indication of the differing plant species life histories, soil types, fertilization or livestock rates than Trangie.

**Grasshopper intake target regulation**

Spatiotemporally, all grasshopper populations, except for the *C. terminifera* Coonamble population, consumed a consistent amount of protein. The demand for protein may remain relatively constant because it is important for maintaining and repairing body tissues as compared to carbohydrate consumption (Harrison et al. 2012). On the other hand, carbohydrates and lipids are
importance for providing animal energy requirements (Chapman 1998). Since energy demand will vary in response to changing activity levels (Arrese and Soulages 2010), carbohydrate consumption will likely be based on environmental cues and life history traits. For example, *Locusta migratoria* (Linnaeus, 1758) individuals that conducted a long distance flight of 120 min significantly impacted the carbohydrate but not the protein consumption compared to inactive individuals (Raubenheimer and Simpson 1999).

We found that grasshopper intake targets varied between populations but were consistently carbohydrate biased and never had an intake target ratio more protein biased than P1:C1. This suggests that their demands for carbohydrates never exceeded their demands for protein. This is congruent with previous studies that found many locust or swarming grasshopper species are carbohydrate biased overall (Cease et al. 2012, Le Gall et al. 2019a, b), in contrast to the nitrogen limitation (e.g., protein biased) paradigm that fits for many herbivores (Mattson 1980, Scriber 1984, White 1993). However, the intake targets in this study were short (three days long) and could indicate a redressing of a nutrient imbalance. Due to this, further research is needed to understand the trade-offs between animal nutrient intake plasticity in relation to a given environment and physiological demands (e.g., development vs. migration).

Adaptations to nutritionally variable environments

Environmental variability influences responses at the individual organism to the population levels (Lande 1993). There are many approaches for accommodating changes in nutritional landscapes which are likely life history specific. Migrations are hypothesized to play an important role in the overall persistence of many animals in variable environments (Southwood 1962), like the arid interior of Australia (Morton et al. 2011). In two of the three populations, *C. terminifera* intake targets remained constant and when compared to the shift seen in *A. thalassinus*, it was largely negligible (Appendix S1: Figs. S2, S3). The Coonamble *C. terminifera* population had an equal nutrient ratio but consumed more protein and carbohydrate altogether (e.g., total nutrient consumption increased) as compared to Trangie and Ivanhoe. Since the Coonamble nutritional landscape matched this species’ self-selected nutrient ratio, it suggests that when times are good, this species will eat...
A similar trend of holding the intake target constant was seen in *O. australis*; however, we did not find a population of this species in a nutritionally suboptimal location nor was a temporal change tested. Not all grasshoppers are capable of long migrations and therefore they may have different coping strategies. *A. thalassinus* is thought to be less migratory and when confronted with suboptimal landscapes they shifted their intake target. This suggests that they do not have the capacity to migrate across landscapes to balance their diet and are redressing a nutritional imbalance. Whereas *C. terminifera* even in the same landscape (Trangie) at two different time points held their intake target constant. This indicates that they may rely more on migration to acquire nutrients as compared to redressing a nutrient imbalance locally. However, we only investigated the intake targets of three species and the results could have been due...
to other life history traits. We hope that this work will spur more comprehensive studies in the future. Further, investigating the post-ingestive regulation abilities of *C. terminifera* will likely illuminate how this migratory species deals with nutritionally suboptimal landscapes.

Nevertheless, there may be foraging trade-offs between migration and post-ingestive regulation to achieve an optimal balance of nutrients. There has been much research regarding habitat stability and the evolution of migratory species and phenotypes (Roff 1994, Dingle 2014). While there has been some consideration in optimal foraging theory and nutritional ecology (Raubenheimer and Simpson 2018), few have looked at the migratory trade-offs in terms of balancing nutrients. Migration is energetically intensive, and in variable environments, there is no guarantee that the animal or population will end up in a nutritionally optimal landscape. In environments with a high degree of rainfall variability, opportunities for follow-up rainfall at the same location and subsequent pasture growth may be rare, making it a challenge for an animal to meet nutritional needs. Comparative nutritional studies between migratory and non-migratory animals may reveal different coping strategies for living in variable nutritional environments.

**Nutritional landscapes within metapopulations networks**

At larger scales, the landscape structure may ensure the persistence of a species within variable environments. At this scale, there are many sink (e.g., suboptimal nutritional landscape) and source (optimal nutritional landscape) habitats, coined the metapopulation (reviewed in Hanski et al. 1997). A strong network of these habitats can lead to the overall persistence of a species. For example, even with extinction-prone local populations of two predator/prey protists species, they are able to persist in a metapopulation structure (Holyoak and Lawler 1996a, b). The total number of patches and interconnectivity of the metapopulation structure is important for the overall persistence of organisms (Wu et al. 1993, Hill et al. 1996, Hanski 1998). Thus, an insect herbivore species may be able to obtain the required nutrients in variable environments at larger scales.

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Table 4. Generalized additive model results for the temporal change of macronutrient consumption (carbohydrate and protein) for *Chortoicetes terminifera*.

| Macronutrient | Variable     | Estimate | SE  | Z     | P      |
|---------------|--------------|----------|-----|-------|--------|
| Carbohydrates | Intercept    | 0.126    | 0.028| 4.454 | ≤0.001 |
|               | Time         | -0.017   | 0.010| -1.759| 0.079  |
|               | Sex          | -0.062   | 0.016| -3.922| ≤0.001 |
|               | Treatment    | 0.008    | 0.008| 1.061 | 0.289  |
|               | Weight       | -0.133   | 0.054| -2.473| 0.013  |
| Protein       | Intercept    | 0.047    | 0.012| 3.825 | ≤0.001 |
|               | Time         | -0.004   | 0.004| -1.057| 0.291  |
|               | Sex          | -0.021   | 0.007| -3.101| 0.002  |
|               | Treatment    | 0.002    | 0.003| 0.675 | 0.499  |
|               | Weight       | -0.037   | 0.024| -1.579| 0.114  |

*Note:* SE, standard error. Models were selected via AIC and can be seen in Appendix S1: Tables S3–S5. Bold values indicate significance.
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