

# Trophic trait plasticity in response to changes in resource availability and predation risk

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## Summary

1. Animals can mitigate the consequences of conflicting food-web constraints (e.g. resource availability vs. predation risk) through plasticity in the expression of functional traits. Because functional trait expression in turn determines a species' impact on ecosystem functions, e.g. its trophic function, there is a growing need to develop a predictive theory that links trait plasticity to ecosystem functioning. But there is currently an incomplete empirical foundation on which to develop such theory.

2. To address this issue, we explored the link between plasticity in a suite of traits (e.g. head morphology, gut size, bite size) that determine the trophic function of a generalist grasshopper herbivore facing predation risk. We reared grasshoppers in the field under two single-forage environments where the plant resources had different physical and nutritional properties and in intermediate mixed-forage environments with or without risk of spider predation.

3. We found that in the single-forage environments, grasshoppers became more efficient at ingesting the resource they were reared on.

4. In mixed-resource environments, grasshoppers developed a phenotype that was not intermediate to the phenotypes in the two single-forage environments, regardless of predation risk.

5. We propose that the suite of phenotypic changes observed in our study represent two alternative feeding strategies that vary with the possibility of using behavioural resource selection in different environmental contexts.

6. Our results suggest that plasticity in trophic function may not emerge from a direct link between the primary traits that determine consumer consumption efficiency given the physical properties of its resources (as is customarily presumed) but rather indirectly from secondary traits that may remain latent until such time that they are drawn into play when the species faces a particular challenge. This means that fully understanding how functional traits influence a species' role in an ecosystem requires consideration of a broader suite of traits than simply those assumed to constrain resource ingestion.

**Key-words:** ecological stoichiometry, feeding morphology, foraging behaviour, functional traits, phenotypic plasticity, predator–prey interactions, stress physiology, trophic function

## Introduction

The nature of a species' impact on an ecosystem ultimately depends on morphological, physiological and behavioural traits that determine the way it functions (Chapin *et al.*

1997; Chalcraft & Reseraris 2003; Naeem & Wright 2003; Hooper *et al.* 2005). Emerging conceptions of the link between functional traits and ecosystem function often presume that such traits are expressed in the same way under different environmental contexts (McGill *et al.* 2006; Petchev & Gaston 2006; Wright *et al.* 2006; Violle *et al.* 2007). But this assumption is at odds with evidence that many traits exhibit context-dependent plasticity (Agrawal 2001; Miner *et al.* 2005; Fordyce 2006; Ghalambor *et al.* 2007; Schmitz 2010). Thus, resolving whether and how environmental context changes trait expression and in turn ecosystem functioning remains an important frontier in the development of a predictive theory for biological diversity–ecosystem function relationships (Schmitz 2010).

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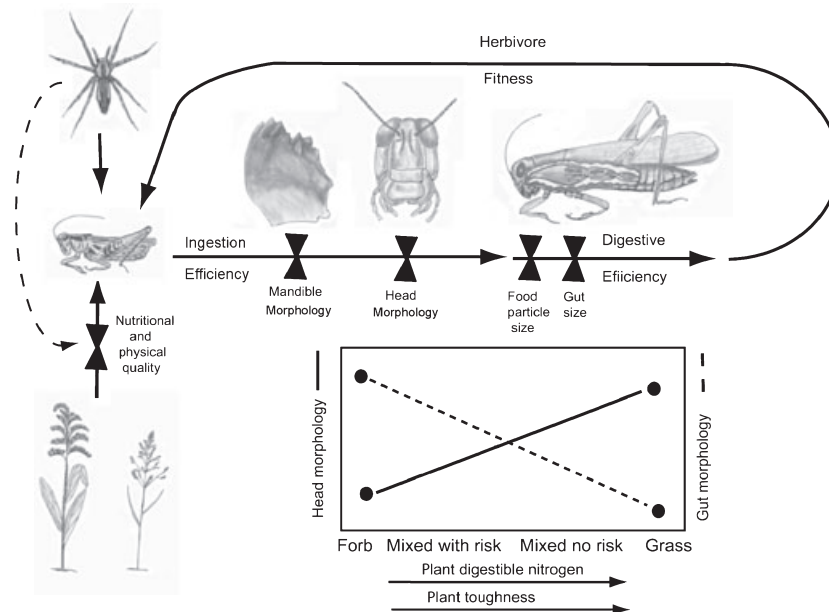
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Gaining such understanding is especially relevant for predicting the functional role of herbivores because, by occupying intermediate trophic levels in food chains, they are simultaneously consumers of resources and resources for other consumers. Herbivores must routinely balance an adaptive trade-off between maximizing foraging gains and minimizing mortality caused by predation (Schmitz 2008b). The degree to which they succeed at this trade-off often depends on their ability to exhibit plastic changes in traits associated with resource consumption as well as predator avoidance (Agrawal 2001; Miner *et al.* 2005; Fordyce 2006; Cressler, King & Werner 2010). There are at least two alternative ways that organisms may deal with such a trade-off, which carry different implications for trophic functioning. First, trait plasticity that improves escape from predation, such as behavioural avoidance of risk, may push individuals into conditions in which their feeding morphology or physiology makes them inefficient at acquiring resources (Olsson & Eklov 2005; Relyea 2005; Eklov & Svanback 2006; Ghalambor *et al.* 2007). Organisms may work within such constraints by producing an intermediate phenotype that balances the conflicting demands in ways that help to reverse the loss of efficiency caused by the initial behavioural switch (e.g. Iwami, Kishida & Nishimura 2007). Such a response would merely modulate the organism's trophic function. Alternatively, an organism may overcome the constraint altogether by expressing an emergent phenotype that in the extreme may lead to an altogether different functional role in the ecosys-

tem, e.g. switching from being a pelagic to a benthic consumer (Eklov & Svanback 2006). Clearly, these represent end points of a continuum of possible responses, and the empirical challenge in functional trait analyses is to discern the extent to which phenotypic plasticity merely modulates the level of functioning vs. altering the nature of functioning among different contexts.

To this end, we present here a test for context-dependent plasticity in a suite of functional traits of a grasshopper herbivore (*Melanoplus femurrubrum*) related to its trophic function (Fig. 1). This generalist herbivore is known to mediate top-down effects of predators on ecosystem properties like plant dominance and diversity and functions like trophic transfer efficiency and nutrient cycling (Schmitz 2003, 2006, 2008a; Hawlena & Schmitz 2010a). This alteration of ecosystem-scale properties and functions is associated with shifts in grasshopper-foraging behaviour in response to elevated risk of spider predation (Rothley, Schmitz & Cohon 1997; Hawlena & Schmitz 2010a). What remains uncertain is whether this is merely a behavioural switch, or if there are associated morphological adjustments that alter grasshopper trophic functioning.

Previous laboratory research with this and a related species demonstrates the potential for plasticity in consumptive traits (e.g. mouthpart and gut morphology) in response to changes in food quality (Thompson 1992; Yang & Joern 1994). But these studies constrained grasshoppers to single-resource diets and did not expose them to predation risk. We, therefore,



**Fig. 1.** A conceptual framework describing adaptive phenotypic responses to changes in generalist grasshopper (*Melanoplus femurrubrum*) diet. Diet composition is based upon resource availability (bottom-up), but is regulated by antipredatory responses to risk of spider (*Pisaurina mira*) predation (top-down). To minimize adverse fitness consequences of coerced changes in diet composition, grasshoppers can improve nutrient absorption by altering morphological and behavioural traits that regulate (X) ingestion efficiency (mandible and head morphology) and digestive efficiency (food particle and gut size). Phenotypic responses for a single-resource diet are based on previous insights (e.g. Thompson 1992). We predict that traits of grasshoppers reared in mixed-forage environment should be intermediate to those induced by the two extreme single-species-rearing environments. Because predation risk is expected to increase the proportion of forb in the grasshopper diet relative to grasshoppers reared on mixed forage in risk-free conditions, we predict that traits of grasshoppers reared in mixed-forage environments with chronic risk should be intermediate to those of grasshoppers from forb-only environments and from mixed-forage conditions without predation risk.

expand on these studies to explore trait plasticity in grasshoppers reared under predation risk in field environments that offered the potential for resource choice. Specifically, we tested whether grasshoppers mitigate consequences of predator-induced dietary constraints by altering quantitative expression of consumptive traits.

## Materials and methods

### PREDICTIONS AND STUDY DESIGN

We conducted our study in a meadow ecosystem in the Yale-Myers Research Forest in north-eastern Connecticut, USA. *Melanoplus femurrubrum* grasshoppers in our field site naturally feed on a mixed diet of grasses (91% of diet) and the dominant forb *Solidago rugosa* (9%). Under risk of spider predation, grasshoppers alter their diet to consume relatively higher proportion of forbs (77%) (Schmitz, Beckerman & Obrien 1997). Thus, we assumed that for *M. femurrubrum* grass is nutritionally more beneficial than forbs. Previous laboratory studies showed that when reared on a single-resource diet, grasshoppers alter their head and mandible morphology with the physical toughness of food, i.e. soft forbs vs. hard grasses (Thompson 1992). Those changes were associated with improved ingestion efficiency of the resource the grasshoppers were raised upon. Additionally, previous studies showed that grasshoppers reared on forage with low nutritional quality (i.e. high C/N ratio) had bigger gut size (Yang & Joern 1994). Based on these results and on previous experimentation in this system, we predicted that a natural mixed-forage-rearing environment should lead to mouthpart, gut size and feeding performance that are intermediate to those induced by grass or forb single-forage environments (Fig. 1). We further expected differences in phenotypes between risky and risk-free contexts that were commensurate with the proportional change in the use of grass and forbs between these contexts (i.e. grasshoppers facing risk consume more forbs than risk-free grasshoppers; Rothley, Schmitz & Cohon 1997). Alternatively, if the phenotypic response is not a collective expression of incremental changes in individual traits but a synchronized multi-trait response to deal with increased predation, then we might expect to see changes in individual traits that diverge substantially from a simple intermediate response to dietary composition.

We tested these predictions in four sequential steps. (i) To reveal differences in forage quality, we compared physical and nutritional characteristics of grass vs. forbs and measured grasshopper assimilation efficiency of N from these resources. (ii) To compare our field results with previous laboratory studies, we reared grasshoppers in the field with exclusively grass or exclusively forbs resources. We then measured grasshoppers' consumptive trait (head and mandible morphology and relative gut size) expression and feeding performance. (iii) To evaluate how grasshoppers mitigate consequences of predator-induced dietary constraints, we reared grasshoppers in the field under natural-rearing environments of mixed forage with and without risk of spider predation and compared their consumptive trait expressions and feeding performance. (iv) We tested whether the phenotypic responses to risk-free and risky mixed-forage environments are a direct result of changes in diet composition by calculating the expected changes in individual traits as the average of the trait measured in the two extreme single-resource diets weighted by the proportions of grass that grasshoppers consume in risk-free and risky environments. Additionally, we evaluated the degree to which grasshoppers can mitigate consequences of dietary constraints by

comparing grasshopper fitness-related traits (body size, denticle erosion, development and body C/N ratio; see Appendix S1) between the four rearing environments (Supporting Information).

### QUANTIFYING PLANT QUALITY FOR GRASSHOPPERS

We quantified the quality of leaves of the dominant grass *Poa pratensis* and the forb *S. rugosa*, which are the main food resources of *M. femurrubrum*, with commonly used measures of physical toughness and nutritional quality (i.e. nitrogen content and assimilation efficiency). We chose to focus on these measures out of a large number of additional factors (e.g. allelochemicals, water content, cell wall structure) known to affect plant quality, because previous research with this and related species examined the grasshopper phenotypic responses to alteration of these specific plant characters (Thompson 1992; Yang & Joern 1994).

We used a simple penetrometer (Sanson *et al.* 2001) to measure an index of leaf toughness, viz. the compression force needed to fracture freshly cut leaves that were tightly straightened in a matching base. We supplemented this physical index of leaf toughness with a functional assay in which we measured ingestion efficiency of *Poa* and *Solidago* leaves by wild-caught grasshoppers from our field site.

We freeze-dried the *Poa* and *Solidago* leaves for 48 h and measured their carbon (C) and nitrogen (N) quality using a C/N auto-analyser. Plant C/N ratio is a common index used to imply plant nutritional quality. However, a C/N ratio provides only an incomplete measure of the plant's true nutritional quality for a herbivore. This is because plants contain nondigestible C-based compounds and may include allelochemicals that temper assimilation efficiency of digestible nutrients. Thus, we complemented our assessment of plant nutritional quality by measuring the approximate digestibility (AD = [dry mass of food digested - dry mass of faeces]/dry mass of food digested × 100 (Nation 2008)) of *Poa* and *Solidago* leaves by *M. femurrubrum* and by calculating the relative assimilation of N from these two plant sources. Feeding trials were preceded by 24 h of food deprivation (water was available) to be sure that grasshoppers egested all faeces derived from foraging in the field. We weighed and placed either four intact blades of fresh-cut *Poa* grass or four intact leaves of fresh-cut *Solidago* into a chamber with an individual grasshopper. After 2 h, we removed all plant material from the chambers and weighed the remains of the blades/leaves. Mass changes were corrected for water loss during the feeding trial. We freeze-dried all leaves for 48 h, weighed them and measured their carbon and nitrogen content using a C/N auto-analyser. To account for possible variation in leaf quality between leaves and even between different parts of an individual leaf, we measured C and N contents only from leaf tissue immediately surrounding the region consumed by grasshoppers. Two days after removing the plant material from the grasshopper chambers, we collected all grasshopper faeces, weighed them and freeze-dried them for 48 h. We weighed the dried faeces and measured their nitrogen content using a C/N auto-analyser. To estimate the amount of N absorbed from *Poa* and *Solidago* per unit dry mass consumed, we subtracted the absolute quantities of N in faeces from that in the plant material consumed.

### REARING ENVIRONMENTS

We created the four rearing environments using cylindrical mesocosms (0.25 m<sup>2</sup> basal area × 1 m high) that were placed over naturally growing vegetation. We created nine blocks of four mesocosms. In each block, we placed one mesocosm over a homogenous patch of

grass, another over a homogenous patch of the forb *S. rugosa*. We placed the two other mesocosms over mixed vegetation composed mainly of grass and *Solidago*. Before stocking the mesocosms with predators and grasshoppers, we manually uprooted the few forb seedlings from the grass-only mesocosms and uprooted all grass from the *Solidago* mesocosms. We repeated this procedure 2 weeks after all cages were stocked. To avoid biasing outcomes because of human disturbance, we also reached into the mixed-forage treatments in the same way as the single-resource treatments but did not uproot plants.

We captured, using sweep nets, second instar *M. femurrubrum* grasshopper nymphs and randomly assigned seven individuals to each of the 36 mesocosms. We used those field-reared grasshoppers for all measurements described in this study. One day later, we added one adult sit-and-wait *Pisaurina mira* spider to one randomly assigned mesocosm of mixed vegetation in each block. Previous research in this system revealed that the presence of one spider is enough to induce substantial physiological and behavioural responses in their grasshopper prey (e.g. Schmitz, Beckerman & O'Brien 1997; Hawlena & Schmitz 2010a). We ensured that the observed trait changes were caused by plasticity in response to predation risk as opposed to size-selective predation, by gluing together spider chelicerae (killing mouthparts) with nontoxic, quick-drying cement. This amendment does not change spider activity, and grasshoppers do not seem to distinguish between manipulated and unmanipulated live spiders (Schmitz, Beckerman & O'Brien 1997). We recovered live spiders from all risk-treatment mesocosms at the end of the experiment.

#### MEASURING GRASSHOPPER INGESTION EFFICIENCY

Four weeks after stocking the mesocosms, we collected grasshopper nymphs and immediately transferred them to individual  $7 \times 2 \times 15$  cm clear plastic chambers in the laboratory according to the block and cage of origin. We measured *Poa* and *Solidago* ingestion efficiency (i.e. intake rate) using Thompson's (1992) adjusted protocol. Grasshoppers from each block were measured simultaneously to reduce variation arising from differences in experimental conditions between days. We alternately assigned grasshoppers from each treatment to eat either *Poa* or *Solidago*. Feeding trials were preceded by 16 h of food deprivation (water was available). Grasshoppers were presented with one preweighed, fresh-cut blade of *Poa* or one fresh-cut *Solidago* leaf. We observed the grasshoppers continuously for up to 1 h and measured the actual time feeding using stopwatches. We removed the blade/leaf from the chamber exactly after 300 s of feeding and reweighed all blades/leaves at the end of the 1-h trial. We calculated the mass of plant material consumed per second during the actual time grasshoppers were observed feeding. We corrected for mass change caused by water loss by calculating a coefficient of water loss because of water evaporation during the feeding trials. We calculated this coefficient by weighing 10 *Poa* blades and 10 *Solidago* leaves (collected from the same plants) before and after each feeding trial. These blades/leaves were placed in identical chambers to those used for feeding trials.

#### MEASURING MORPHOLOGICAL TRAITS AND BITE SIZE

We measured the effects of rearing environment on grasshopper body and mouthpart morphology, gut size, bite sizes and fitness-related traits by sacrificing grasshoppers after laboratory feeding trials and by sacrificing additional grasshoppers from the field mesocosms. All grasshoppers were photographed using a Leica MZ12.5 stereomicroscope ( $\times 20$ ) (Leica Microsystems, Wetzlar, Germany), and images

were displayed using IMAGEJ software (National Institutes of Health, Bethesda, Maryland, USA). We then measured the pronotum, tibia, femur and four head morphological traits: head length – the distance from the top of the fastigium to the middle of the epistomal suture viewed from the front, head width – the distance between the widest points of the epicranium when viewed from the front, anterior articular width (AAW) – the distance between the anterior articulation points of the mandibles and posterior articular width (PAW). These measurements were chosen because they quantify aspects of head morphology that may be important in feeding performance (Thompson 1992).

Using a scalpel under a dissecting microscope, we removed the grasshopper's left mandible and gut. We evaluated the effect of rearing environment on mandible morphology using characters identified by homologous reference points (Patterson 1983). We used a Keyence 600E 3D digital microscope ( $\times 150$ ) (Keyence Corporation, Osaka, Japan) to measure four mandible characters: the chordal length from the anterior articular process of the mandible to the second molar dent (AP-MD), the chordal length from the posterior articular process of the mandible to a tangent connecting the second and third incisive dents (PP-ID) and the chordal length between anterior and posterior articular processes (AP-PP). We also evaluated the effect of rearing environment on the mandible denticle erosion by measuring the relative heights of the incisor and molar apices from a perpendicular line connecting the second and third incisive dents (Appendix S1, Supporting Information). We used an auto-depth composition function to reduce measurement imprecision because of edge deviation and magnification fluctuations caused by the shifts of focus position.

We evaluated the effect of rearing condition on grasshopper bite size by removing the crop content, smearing it on a microscope slide and fixing it with a drop of Karo corn syrup. We measured the length of 25 pieces of *Poa* and *Solidago* in 5–8 microscope fields ( $\times 150$ ), using a Keyence 600E 3D digital microscope. Preliminary 'collector curve' measurements showed that this sample size is sufficient to account for variation in food particle sizes. We used the average *Poa* and *Solidago* piece size for each slide as an independent sample for all analyses.

We freeze-dried the empty gut and the rest of the body separately for 48 h before obtaining the dry mass of each. We calculated the proportion of gut dry mass to body dry mass (G/B; Yang & Joern 1994) to evaluate the relative allocation to digestive tract in response to the rearing environments.

## Results

#### RESOURCE QUALITY

Using univariate general linear models (GLM), we found that the leaf toughness index of *Poa* ( $73.45 \text{ g mm}^{-2}$  95% CI 64.57–83.75) was 1.57 $\times$  higher ( $F_{1,48} = 25.86$ ;  $P < 0.001$ ) than that of *Solidago* ( $46.24 \text{ g mm}^{-2}$  95% CI 40.64–52.60). Grasshopper feeding efficiencies on *Poa* blades and *Solidago* leaves revealed even stronger differences than the physical leaf measurements suggest. Grasshoppers consumed *Solidago* leaves 2.97 $\times$  quicker than *Poa* blades (GLM;  $F_{1,101} = 132.04$ ;  $P < 0.001$ ). There was no statistical difference (GLM;  $F_{1,63} = 3.10$ ;  $P = 0.083$ ) in the absolute C/N ratio between *Poa* blades ( $19.61 \pm 6.4$ ) and *Solidago* leaves ( $18.08 \pm 0.59$ ). However, using analysis of covariance, we found that grasshoppers assimilated 13 $\times$  times more N per unit dry mass



consumed when eating *Poa* than eating *Solidago* ( $F_{1,33} = 5.701$ ,  $P = 0.023$ ). Thus, *Solidago* appears to be easier to ingest than *Poa* but provides grasshoppers less N per unit dry mass consumed, supporting our prior expectations that *Poa* is a better source of N for grasshoppers.

#### PHENOTYPIC RESPONSES IN THE TWO SINGLE-RESOURCE ENVIRONMENTS

Quantitative morphological measures and feeding performances are allometrically related to body size. Because we were interested in changes in shape, relative size or relative performances, morphological and performance measurements were analysed with body size as a covariate. As in other studies of insect morphology, tibia length was used as a body size covariate because it is not affected by rearing environment. Using a mixed-model GLM with block as a random factor, we found a significant interaction in ingestion efficiencies between rearing environment and type of food presented in the laboratory feeding trials ( $F_{2,40} = 34.67$ ,  $P < 0.001$ ; Fig. 2). These results mirrored previous laboratory results (Thompson 1992). However, using multivariate analysis of covariance (MANCOVA), we found no statistical difference between grass and forb-rearing environments in grasshopper head morphology (Pillai's Trace = 0.164;  $F_{4,51} = 2.496$ ;  $P = 0.054$ ) or mandible structure (Pillai's Trace = 0.0734;  $F_{3,39} = 1.028$ ;  $P = 0.391$ ) that could account for the variation in ingestion efficiency. We did not find differences in bite size between grass and forb-rearing environment when grasshoppers were fed on *Solidago* (GLM;  $F_{1,12} = 0.015$ ;  $P = 0.904$ ), but did find significant differences when grasshoppers were fed *Poa* (GLM;  $F_{1,5} = 8.442$ ;  $P = 0.034$ ). Grasshoppers from grass-only environments ingested bigger *Poa* pieces than grasshoppers reared in forb-only environments. Finally, we did not find differences in relative gut size

between grass and forb-rearing environments (GLM;  $F_{1,55} = 1.813$ ;  $P = 0.184$ ).

#### PHENOTYPIC RESPONSES IN RISKY AND RISK-FREE MIXED-DIET ENVIRONMENTS

Risk of spider predation did not affect grasshopper morphological traits related to feeding and did not affect feeding performance. Grasshopper ingestion efficiencies did not differ between risk-free and risky mixed-forage environments (GLM;  $F_{1,55} = 0.867$ ,  $P = 0.356$ ), after correcting for grasshopper body size. We did not find differences in grasshopper head morphology (MANCOVA; Pillai's Trace = 0.112;  $F_{4,54} = 2.013$ ;  $P = 0.103$ ) or mandible morphology (MANCOVA; Pillai's Trace = 0.012;  $F_{3,44} = 0.174$ ;  $P = 0.913$ ) between grasshoppers reared in risk-free or risky mixed-forage environments. We did not find differences in bite size between risk-free and risky mixed-forage-rearing environments when grasshoppers were fed on *Solidago* (GLM;  $F_{1,17} = 0.508$ ;  $P = 0.485$ ) or *Poa* (GLM;  $F_{1,4} = 0.558$ ;  $P = 0.496$ ). Finally, we did not find differences in relative gut size between risk-free and risky mixed-forage-rearing environment (GLM;  $F_{1,61} = 1.008$ ;  $P = 0.319$ ).

#### EXPECTED VS. OBSERVED PHENOTYPE OF GRASSHOPPER REARED IN MIXED-FORAGE ENVIRONMENTS

We tested whether the phenotypic responses to risk-free and risky mixed-forage environments are a direct result of changes in diet composition by calculating the expected changes in individual traits as the average of the trait measured in the two extreme single-resource diets weighted by the proportions of grass that grasshoppers consume in risk-free (0.91) and risky (0.325) environments (Schmitz, Beckerman & O'Brien 1997). Using t-tests to compare the observed and expected values, we found that grasshopper nymphs reared in risk-free and risky mixed-forage environments developed phenotypes that differ from the intermediate phenotypes expected based on the diet composition (Table 1). Predominantly, grasshoppers reared in mixed-forage environments developed relatively larger heads, mandibles and guts, and had relatively larger bite sizes than expected based on diet composition (Fig. 3b–d). These changes corresponded with a food ingestion rate that was higher than expected based on diet composition (Fig. 3a).

#### Discussion

Our objective was to assess whether *M. femurrubrum* grasshoppers demonstrate context dependency in the expression of their trophic traits and the extent to which any potential change was the sum of incremental responses of individual traits or a synchronized multi-trait response to accommodate a new environmental challenge. We built on earlier benchmarks (Thompson 1992; Yang & Joern 1994) that resource quality could alter trophic traits in this species, when individuals were constrained to use single diets of either tough

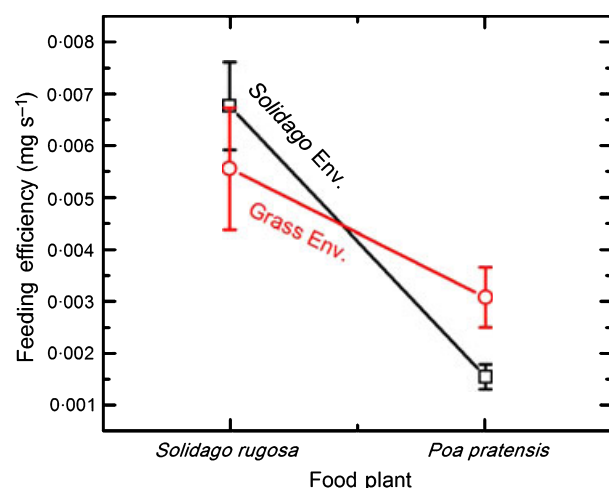


Fig. 2. Ingestion rate in milligrams of dry mass per second (adjusted for body size)  $\pm$  SE of *M. femurrubrum* when feeding on either *Poa pratensis* or *Solidago rugosa* (horizontal axis). The two different lines connect grasshoppers that were raised in *S. rugosa* or Grass spp. single-forage environments. Note the overall higher consumption rate on *S. rugosa*.

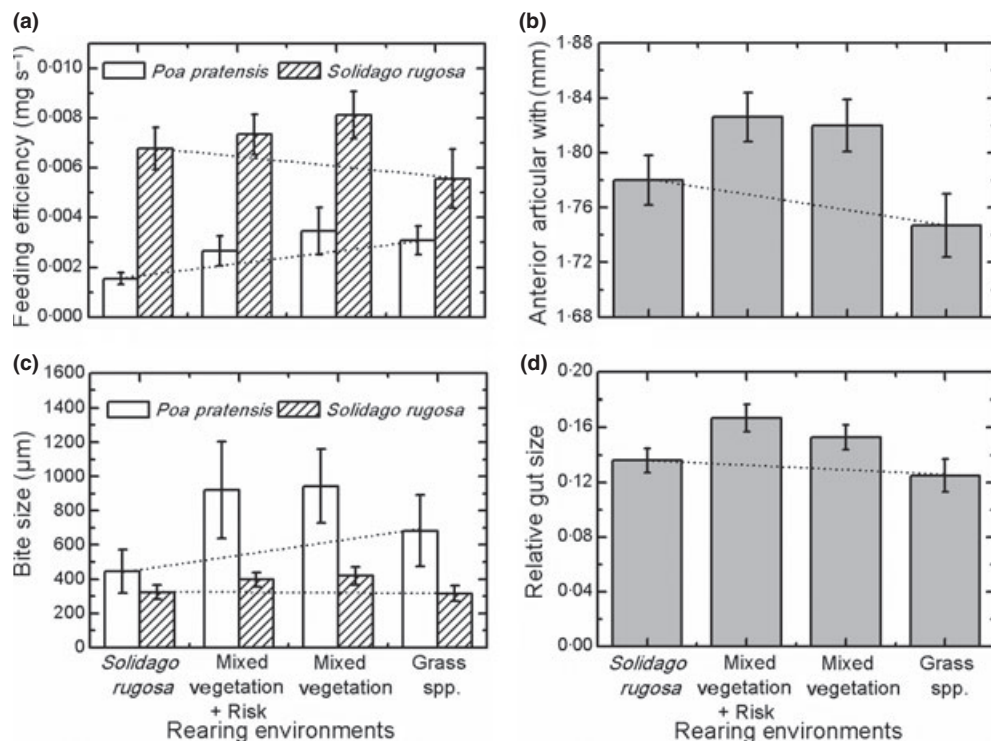
**Table 1.** Results of t-tests comparing the observed trait expressions of grasshoppers reared in mixed-forage environments with (risky) or without (risk-free) disarmed hunting spiders, to the intermediate phenotypes expected based on diet composition

	Risk-free			Risky		
	<i>t</i>	d.f.	<i>P</i>	<i>t</i>	d.f.	<i>P</i>
<b>Ingestion efficiency</b>						
<i>Poa</i>	-2.18	16	0.045	-3.35	15	0.004
<i>Solidago</i>	-3.37	11	0.006	-2.975	15	0.009
<b>Head morphology</b>						
Head width	-2.67	44	0.011	-5.51	34	0.001
Head length	-1.12	44	0.269	-2.35	34	0.025
AAW	-4.61	44	<0.001	-4.71	34	<0.001
PAW	-3.3	44	0.002	-4.4	34	<0.001
<b>Mandible morphology</b>						
AP-MD	-2.54	27	0.017	-3.15	28	0.004
PP-ID	-3.51	27	0.002	-3.56	28	0.001
AP-PP	-1.35	27	0.187	0.20	28	0.420
<b>Bite size</b>						
<i>Poa</i>	-3.13	11	0.017	-2.69	14	0.075
<i>Solidago</i>	-3.22	11	0.008	-2.78	14	0.015
<b>Gut morphology</b>						
Size	-2.68	36	0.011	-3.14	33	0.004

AAW, anterior articular width; PAW, posterior articular width; AP-MD, chordal length from the anterior articular process of the mandible to the second molar dent; PP-ID, chordal length from the posterior articular process of the mandible to a tangent connecting the second and third incisive dents; AP-PP, chordal length between anterior and posterior articular processes.

grasses or softer forbs. We found that grasshopper individuals were most efficient at ingesting the single resource on which they were reared, consistent with previous laboratory experiments (Thompson 1992). Consistent with this observation, we also found that grasshoppers from grass-only environments ingested bigger *Poa* pieces than grasshoppers reared in forb-only environments. However, unlike the laboratory studies (Thompson 1992; Yang & Joern 1994), we did not identify morphological adjustments that could explain this 'home-field advantage'.

Phenotypic changes in functional traits can result from collective expression of incremental changes in individual traits, or from synchronized trait responses to accommodate a new environmental challenge. We predicted that if the former pathway governs the change in individual trait expressions, then the response to mixed-forage-rearing environment should be intermediate to the trait expressions of grasshoppers reared in the two single-forage environments. Based on the acknowledged diet shift in response to predation risk (Rothley, Schmitz & Cohon 1997), we also predicted that trait expression of grasshoppers reared in the risky mixed-forage environment should be intermediate to trait expression under risk-free mixed-forage- and *Solidago*-only-rearing environments (Fig. 1). Our results did not support these predictions (Fig. 3). We found that grasshoppers reared in risky and risk-free mixed-forage environments had similar trophic morphologies and ingestion efficiencies. We also found that these



**Fig. 3.** Comparison of grasshoppers' feeding performances and feeding-related traits between the four rearing environments. (a) Variation in ingestion efficiencies, when grasshoppers were experimentally fed on the grass *Poa pratensis* (empty) or the forb *Solidago rugosa* (bars). (b) Variation in anterior articular width (AAW). (c) Particle size (bite size) retrieved from grasshoppers' crop after being experimentally fed grass *P. pratensis* (empty) or the forb *S. rugosa* (bars). (d) Relative gut size. The dotted lines represent intermediate phenotypes that are expected if grasshopper phenotype is a direct reflection of their diet composition and the error bars denote  $\pm 1$  standard error.

phenotypes were not intermediate to trophic morphologies and ingestion efficiencies of grasshoppers reared in the two single-resource environments. There seemed to be a synchronized change in multiple traits that alter grasshopper's trophic morphology and function.

We propose that the suite of phenotypic changes observed in our study (Fig. 3) represent two alternative feeding strategies that vary with the possibility of using behavioural resource selection in different environmental contexts. Herbivore digestion efficiency is effectively determined by the product of three forage attributes: food quality, particle size and forage digesta retention (Vansoest 1996; Clauss *et al.* 2009). When reared in a single-resource environment, herbivores are forced to gain all essential nutrients from a single plant resource. In this case, herbivores have no recourse to increase food quality, so they must either play with particle size or retention time to enhance digestion efficiency (Vansoest 1996). Forage digesta retention time limits the amount of food a herbivore can consume per unit time. Thus, herbivores are most likely to improve digestion efficiency by reducing the size of food particles entering the crop (Clauss *et al.* 2009), which is determined by adjusting bite sizes. Differential release of proteases and carbohydrases in the herbivore gastrointestinal tract can further improve the assimilation of required nutrients (Clissold *et al.* 2010). In contrast, grasshoppers in mixed-forage environments can flexibly adjust their ingestion of different plant resources that provide specific nutrient balances (Bernays 1998; Behmer & Joern 2008). This alleviates the need to extract 'every last nutrient' out of a particular unit of resource. In turn, grasshoppers can increase ingestion efficiency by taking large bite sizes. But this comes with the cost that it is more difficult to extract nutrients from the ingested resource. Grasshoppers may partially compensate by increasing their gut size (Yang & Joern 1994).

However, attempts to reduce the risk of predation may hinder grasshopper flexibility to obtain required nutrients by behaviourally switching between food sources. Thus, one would expect that grasshoppers should develop a compensatory phenotype to minimize the costs involved in restricting the behavioural choice (for details see Supplementary Information). This raises the following question: Why did grasshoppers reared in risky mixed-forage environments not alter their phenotype relative to risk-free mixed-forage environments? One possibility is that morphological changes are less flexible than behavioural changes and would thus commit a consumer to a costly strategy that may interfere with other activities, when predation threat is not present. Thus, consumers may not use morphological changes if predation risk is ephemeral. But we exposed grasshoppers to chronic predation risk, so this explanation for lack of change is less tenable.

An alternative explanation for the morphological patterns derives from the possibility that the change in foraging behaviour is an indirect (rather than direct) consequence of predation threat. The traditional view is that any shift from a preferred diet is assumed to result from constraints on a consumer's ability to acquire food sources that best satisfy nutritional demands. In conventional theoretical models of

trophic interactions (e.g. Abrams 1984; Bolker *et al.* 2003; Krivan & Schmitz 2004), prey resource selection is assumed to be constrained by *direct* antipredator responses such as reduction in feeding and handling time (Hawlena & Pérez-Mellado 2009) and a shift in foraging activity to safer but nutritionally poorer habitats (Abramsky, Rosenzweig & Subach 2002). In this case, prey is expected to overcome the costs of dietary restrictions as a result of antipredator behaviour via phenotypic changes to use less favourable resources more efficiently (Eklov & Svanback 2006). The fact that grasshoppers demonstrated no change between the risky and risk-free contexts in the mixed-forage environments calls for a revision in thinking about the way predation risk might influence trophic roles in herbivore prey.

Explaining the indirect effect requires the additional consideration of herbivore physiology (Hawlena & Schmitz 2010b). Grasshoppers in our study system respond to chronic risk of predation by increasing their basal metabolic rate and by enhancing their escape performances (Hawlena & Schmitz 2010a; Hawlena *et al.* 2011). These responses alter the grasshopper nutritional requirement favouring resources rich in digestible carbohydrate to fuel heightened metabolic costs at the expense of consuming protein-N (Hawlena & Schmitz 2010b). In our system, *Solidago* leaves offer 2.5× more nonfibre carbohydrates ( $55.4 \pm 0.5\%$ ) than *Poa* leaves ( $21.9 \pm 1.8\%$ ). Thus, increased grasshopper use of *Solidago* leaves when exposed to chronic risk of predation may be caused by changes in predator-induced physiological demand for a different nutrient balance rather than a predation-induced constraint. This in turn should lead to higher body C/N ratio than in risk-free conditions. We observed this shift in body C/N content between environmental contexts (Appendix S1, Supporting Information).

Consequently, plasticity in trophic functioning of herbivores may not be determined by constraints in trophic morphology (e.g. mouthparts and gut) customarily examined in the analyses of herbivore phenotypic plasticity. Rather, we may see no morphological changes because grasshoppers should maintain their ability to flexibly choose the diet composition that meets flexible changes in physiological nutrient demands as the predation risk context changes. Accordingly, models of trophic interactions may need to be restructured to appropriately characterize the predation risk effects on herbivore traits and ecosystem functions. Classic models predict the effect of predation risk-foraging trade-offs in terms of restrictions on resource intake (gain function). But if prey responses to predation risk are driven by metabolic demands for nutrients (costs functions), then predation risk effects must be expressed in terms of nutrient contents (i.e. digestible protein-N and carbohydrate-C) of plant resources (Behmer & Joern 2008; Schmitz 2008b; Raubenheimer, Simpson & Mayntz 2009) rather than in terms of resource identity or structural traits that influence feeding, handling and processing time costs.

In conclusion, the trophic role of herbivores in ecosystems may not derive from incremental, quantitative changes in one or more trophic traits in response to a particular challenge



(e.g. resource toughness changes mouthpart morphology). Instead, our results suggest that trophic function emerges from multiple morphological, physiological and behavioural traits whose integrative expression is not correlated with the full suite of changes in environmental context. For example, the effects of changing grasshopper metabolic rate would only be observed between risky and risk-free environments, but not between environments with different resource qualities. The implication is that important traits that may ultimately determine a species' functional role may remain latent until such time that they are drawn into play when the species faces a particular challenge (Schmitz 2010). In our example, constraining grasshopper ability to use behaviour to consume preferable diet leads to phenotypic changes in trophic morphologies and function. This means that fully understanding how functional traits influence a species role in an ecosystem requires consideration of the broader context in which the species exists and in which its traits may have evolved. For many herbivores that have evolved to respond to the dual challenges of selecting nutrients and avoiding predation, it will be insufficient to examine these species within a subset of ecological contexts (e.g. considering only plant–herbivore interactions or herbivore stoichiometry in the absence of predators) in which they are normally found if we wish to fully understand their functional role in ecosystems (Schmitz 2010).

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## Supporting Information

Additional Supporting information may be found in the online version of this article.

**Appendix S1.** Implications of environmental constraints on fitness related traits.

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