Critical decisions: risk allocation in a model arthropod (*Folsomia candida*)

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This thesis has been prepared

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The undersigned certify that they have read, and recommended to the Graduate Studies Committee for acceptance, a thesis entitled "Critical decisions: risk allocation in a model arthropod (*Folsomia candida*)" submitted by Allison Bannister in partial fulfilment of the requirement for the degree of Master of Science.

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Abstract

Risk allocation theory predicts that foraging animals moderate predation risk by allocating their foraging effort and space use according to their energetic demands. Undernourished animals have a greater need for energy than do sated individuals and should accept higher risk while foraging. Original theory predicted that the proportion of time that individuals spend in good conditions primarily determines risk allocation. More recent theory predicts that the length of exposure to good or bad conditions governs risk allocation decisions when patterns of environmental risk are autocorrelated in time. I investigate the effects of these factors with controlled experiments on a standard arthropod (*Folsomia candida*). I subjected animals to nine temporally autocorrelated 16-day feeding treatments varying in both the proportion (0, 0.25, 0.50 and 0.75) and duration (short, medium and long intervals) of time when food was absent. Risk allocation was assessed by the choice of occupying a risky dry habitat where food was present (rich) versus a safe moist habitat with no food (poor). Irrespective of autocorrelation in conditions, the proportion of time spent with no food primarily determined risk allocation by these Collembolans. The results suggest an energetic threshold below which *F. candida* are forced to forage in rich and risky habitat despite the possibility of mortality through desiccation. State dependent patterns of habitat selection suggest that understanding the relationship between energetic state and patterns of environmental condition may allow us to employ risk allocation as a leading indicator of habitat change.

Lay Summary

Faculty and students in the Department of Biology are bound together by a common interest in explaining the diversity of life, the fit between form and function, and the distribution and abundance of organisms. The research reported here demonstrates that the ways in which animals deal with the conflicting demands of food versus safety influences their use of habitat and thus, patterns of distribution and abundance in nature. Theory predicts that an animal's energetic state, and subsequent use of resources and habitat, depends on the temporal sequence of safe and risky conditions to which they have been exposed. I provide the first test of this crucial idea with controlled experiments on small arthropods (Springtails). My experiments varied the frequency and duration of time that populations received food, verified that individuals within the populations were in different energetic states, then assessed their relative use of a safe habitat lacking food versus a risky one with superabundant food. Individuals in populations that received the least food sacrificed safety in order to feed. The results document an important role by which past events account for current behaviour and habitat use. The research also points towards a day when we can use such behaviours to document changes in habitat quality before those changes cause irreversible declines in population density.

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I would like to thank my supervisor Dr. Douglas Morris for his contribution of time, intellectual and financial resources and patient tutelage; my supervisory committee members, Dr. Stephen Hecnar, Dr. Robert Rempel and external examiner, Dr. Brian McLaren, for their invaluable feedback and review; Dr. Gilles Boiteau for providing our lab with stock populations of Collembola; lab members Daniel Durston, Denon Start, Sarah Moreth and MaryJane Moses for their guidance and support; all funding agencies, including NSERC and Lakehead University; and my family, for encouraging me to go forth boldly in the direction of my dreams.

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Introduction

A cornerstone of foraging theory is the recognition that the decisions individuals make while foraging will impact their evolutionary fitness. Choices of where and when to forage can determine whether or not an individual succumbs to predation or starvation during a foraging bout. Foragers adopting an optimal strategy should thus be selective and bias foraging efforts towards resource patches with the most favourable ratio of food availability to risk (Brown 1988; Brown *et al*. 1999; Kotler *et al*. 2010). Such individuals should reduce their feeding effort when predation risk is high and also increase anti-predator behaviours such as vigilance (Kotler *et al*. 2010; Lima and Bednekoff 1999). Conversely, individuals should increase feeding effort when the environment becomes less dangerous (Lima and Bednekoff 1999). These insights into the effects of predation on forager behaviour are described by the predation risk-allocation hypothesis (Lima and Bednekoff 1999) in which the trade-off between vigilance and foraging effort, and its interactions with a forager's energetic state, force hungry animals to accept more risk whenever predators become more active or abundant. A clear understanding of foraging under risk allows us to better comprehend the effects of predation on foraging behaviour, patch use, and habitat selection in temporally fluctuating environments (Sih *et al*. 2000).

Thus far, empirical tests of the risk-allocation hypothesis have yielded mixed conclusions (Ferrari *et al*., 2009; Koivisto and Pusenius 2003; Sih and McCarthy 2002; Sundell *et al*. 2004), that may be due to omissions in the original model. Failure to include autocorrelated environmental conditions in experiments on risk allocation can produce indeterminate results (Higginson *et al.* 2012). If animals are exposed to autocorrelated environmental conditions in their natural environment, but not in

experiments, then experiments are unlikely to detect the animals' optimal allocated vigilance. Thus, the predictions of the original predation risk allocation hypothesis may not accurately represent the behaviour of foragers in natural systems. In order to better predict general patterns of risk allocation, empirical studies should explore the effect of autocorrelated conditions on risk allocation behaviour, and compare tests of the original predictions to those of the modified theory under the same controlled conditions (Higginson *et al*. 2012).

I describe experiments where I expose a small arthropod, the springtail *Folsomia candida*, to different patterns of environmental change. In particular, I manipulate both the autocorrelated proportion of time (p) that animals are exposed to unfavourable conditions as well as the length of those bad conditions (d).

I begin by briefly reviewing risk-allocation theory as well as the life history of *F. candida* as it applies to tests of risk allocation. I outline how I manipulated energetic state, and how I confirmed changes in energetic state by measuring survival and fecundity. I demonstrate how we can use habitat selection as a measure of risk allocation (Fountain and Hopkin 2001; Lima and Bednekoff 2011) and desiccation stress in Collembola as a general risk factor (Edney 1977). I describe the results and discuss their significance to our understanding of risk-allocation behaviour. I conclude by discussing whether such behaviours may enable us to detect changes in habitat before those changes cause populations to decline.

Predicting risk allocation behaviour

The original predation risk-allocation hypothesis (Lima and Bednekoff 1999) models the foraging behaviour of an individual over a specific time interval (T) in which it must attain an energetic threshold (E) in order to avoid starvation (Ferrari et al. 2009; Lima and Bednekoff 1999). T is divided into periods of risky and safe environmental conditions and foragers in this model must allocate foraging between these periods according to both their current energetic state and estimate of future environmental conditions. The theory predicts if periods of high risk are short or infrequent, that optimal foragers should abstain from foraging during these brief pulses, reserving their foraging effort for better future conditions. The optimal foraging effort in high risk (F_H^*) and low risk (F_L^*) conditions is given by Lima and Bednekoff (1999) as

$$F_H^* = \frac{R}{\left(\alpha_H/\alpha_L\right)(1-p)+p} \quad (1)$$

$$F_L^* = \frac{R}{(1-p)+\left(\alpha_L/\alpha_H\right)p} \quad (2)$$

where (α_H/α_L) and (α_L/α_H) represent the respective attack ratios in high risk and low risk conditions. The proportion of time the environment is in a low risk condition is represented by (1- p), and R is the average rate of foraging required to meet E by time T. As the proportion of time spent in risky conditions (p) increases, the ability to reduce risk through vigilance or space use decreases during both safe and risky conditions as

opportunities to forage in safety become rare, and the energetic state of foragers declines (Figure 1; Lima and Bednekoff 1999).

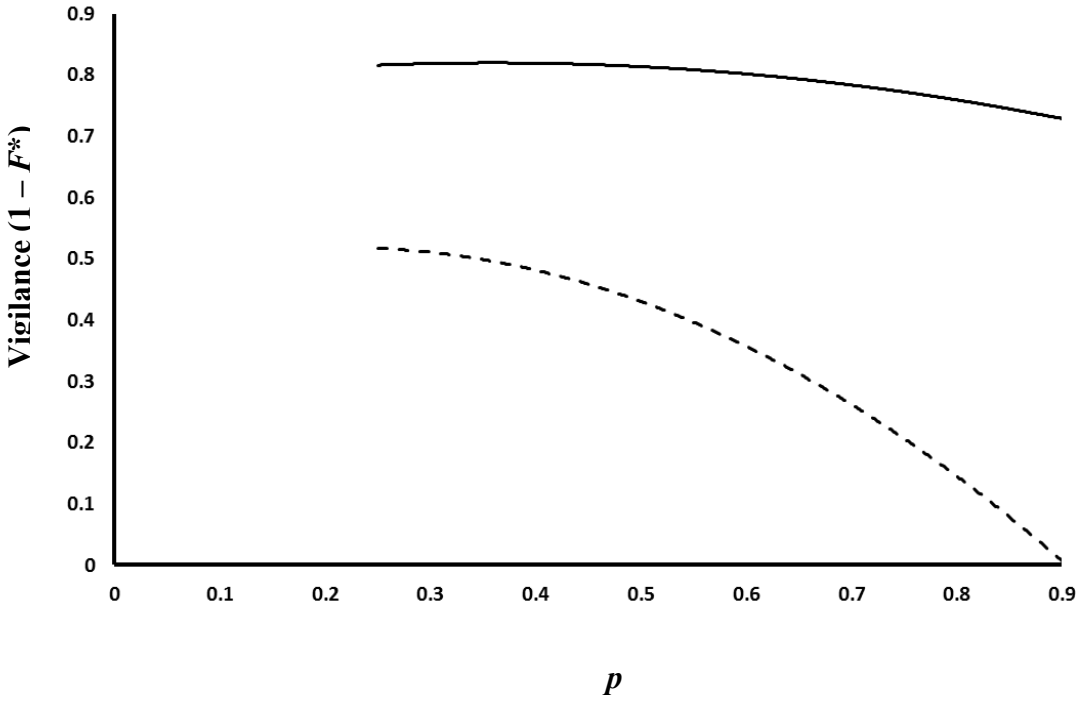

Figure 1: Vigilance as influenced by the proportion of time (*p*) animals are exposed to high risk conditions. When environmental conditions are not autocorrelated in time, vigilance decreases in both safe (dotted line) and risky (solid line) conditions as the proportion of time spent in high risk conditions increases. The average rate of foraging, and the attack ratio in high-risk conditions, are set at 0.4 and 3.0, respectively. A similar pattern emerges for other mechanisms of risk allocation such as habitat selection. Modified from Lima and Bednekoff (1999; Figure 3).

The decision to forage during risky conditions depends on whether the organism's current energetic state is sufficient to sustain the individual until good times reappear (Higginson *et al*. 2012). In environments with no temporal autocorrelation, only the frequency of risky conditions provides information on the probability of encountering safe conditions. When the environment fluctuates predictably, however, organisms

conditioned (e.g., cognitively or physiologically) to those fluctuations obtain more accurate predictions about the future environmental state from the duration of events (*d*), than from *p*. So when safe and risky times fluctuate rapidly, vigilance should increase and animals should forage only during the safe periods (or in rich places). But when safe and risky times fluctuate more slowly (longer durations of each event, and particularly so for risky periods), then less vigilant foraging will increase survival. Similar interpretations hold when the forager's state depends on food rich and food poor intervals of time (Higginson *et al*. 2012).

I illustrate these effects for two environments in Figure 2 ($p = 0.5$). In panel A, the environment switches rapidly between safe and risky conditions, where each condition is present for half of the foraging period. Foragers should show extreme risk allocation, stocking up on food during safe periods and ceasing activity during dangerous periods. Panel B displays the opposite situation where periods of environmental conditions have a long duration. Foragers cannot forego foraging during dangerous conditions because they then risk starving before conditions improve. Panel C depicts a third environment with varying durations of safe and risky conditions. Here, risk allocation behaviour depends on the recent temporal pattern of safety (or food availability).

The usual test of risk allocation is to evaluate vigilance through time as animals are exposed to risky versus safe conditions (Ferrari *et al.* 2009). These assays work reasonably well for organisms that can be easily and effectively observed, or for which assays of risk allocation such as use of safe and risky foraging sites can be collected through time. An alternative is to manipulate the organism's energetic state by varying

the duration and frequency of rich and poor conditions, then assay its use of safe but resource-poor habitat versus its use of risky food-rich habitat.

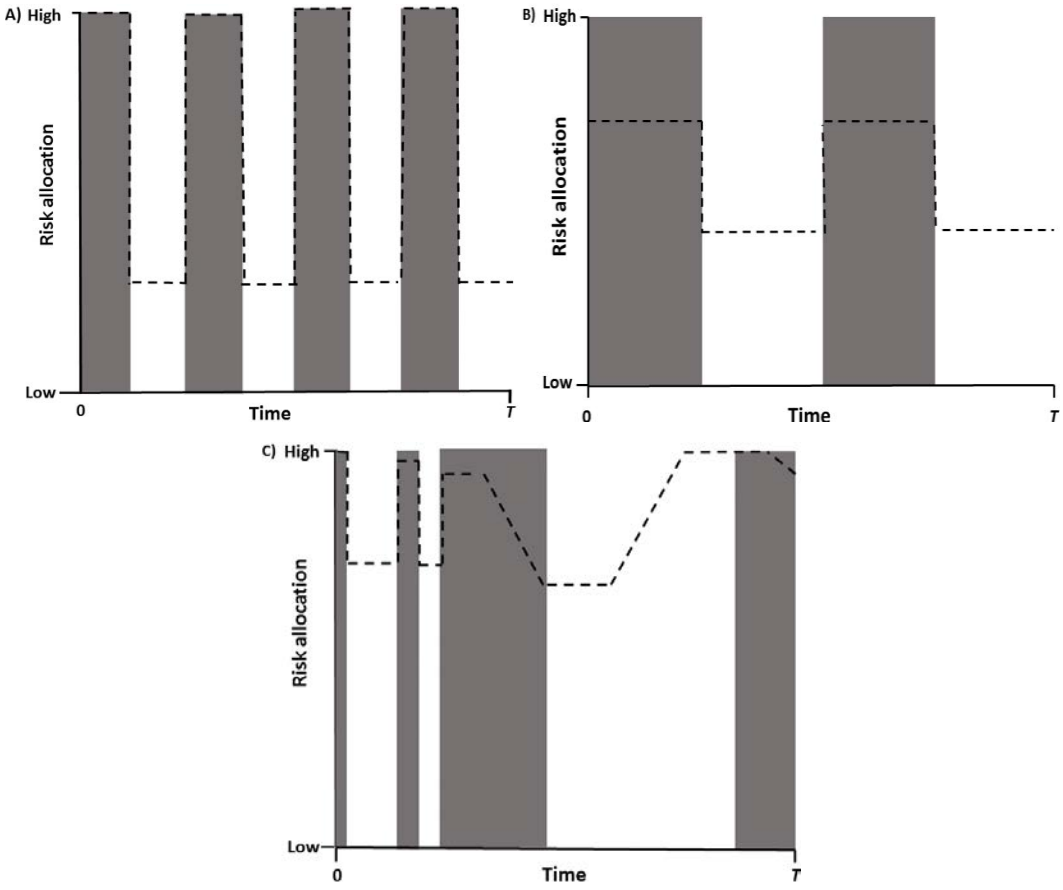

Figure 2: Expected patterns of risk allocation behaviour in three different environments where periods of safe and risky conditions are variously autocorrelated in time such that each condition occurs for 50% of the time. Risky periods are indicated by shaded bars and the amount of expected risk allocation behaviour (low to high) is indicated by dashed lines. A, the environment switches rapidly between conditions. Foragers should demonstrate extreme risk allocation, foraging heavily during safe times and ceasing to forage during risky times. B, the rate of switching between conditions is slow and risk allocation behaviours are reduced because animals cannot forego foraging or else they may starve before conditions improve. C, the pattern of foraging depends on the temporal pattern of conditions and risk allocation behaviour in both conditions decreases as the periods of risky conditions become longer.

In order to appreciate this approach, imagine an environment that fluctuates regularly between rich and poor periods of equal duration (perfect negative

autocorrelation, $p = 0.5$). After conditioning the animals to the environment, they are then given a choice between the two habitats following the final poor period. If the duration of events is short, the animals' energetic state should be high, and individuals should be unlikely to occupy the risky habitat. If the duration of poor periods is long, however, then the animals' energetic state should be low, and they will accept greater risk to obtain food (greater probability of occupying the risky and rich habitat). Now imagine that one varies the frequency of rich and poor periods. Animals living in environments biased towards good periods should, on average, be in a higher energetic state than individuals living in environments dominated by resource scarcity. Such animals can afford to allocate less time to vigilance and foraging in risky places. Thus, in a properly designed experiment we can anticipate that both the proportion and duration of events will interact to determine risk allocation, and hence, habitat selection.

I conduct a test of risk allocation theory that meets these criteria. I manipulate both the duration and proportion of time that a model organism, *Folsomia candida,* is exposed to food rich versus no food conditions. I assess survival and reproductive rates of animals living under the different regimes as surrogate estimates of the animals' energetic state. Animals in a low energetic state should experience lower reproduction and possibly lower survival and offspring quality than animals in a higher state. I complete the experiment by then assaying the habitat choice by Collembola between rich and risky versus poor and safe habitats in order to assess the effect of energetic state on risk-allocation behaviour.

METHODS

Study population

I obtained a laboratory culture of *Folsomia candida* Willem (Collembola: *Isotomidae*) from an established research population at the Agriculture and Agri-food Potato Research Center in Fredericton, New Brunswick, Canada (laboratory of Dr. G. Boiteau). *Folsomia candida* is a globally abundant, parthenogenic, soil-dwelling hexapod (Fountain and Hopkin 2005; OECD 2009). As all individuals within a population can be considered the same, energetic state can easily be tracked through reproduction and survival (Croua and Cazes 2003). *F. candida* are pigmentless and lack external photoreceptors (Fountain and Hopkin 2002, 2005; Fox *et al*. 2005), the absence of which enables habitat choice to be quantified under full light. A more detailed description of the life history of *F. candida* is in Appendix 1.

F. candida is nonetheless highly sensitive to other external stimuli and is easily cultured in the laboratory (Fountain and Hopkin 2005). *F. candida*'s small-body size and absence of a desiccation-resistant cuticle (Fountain and Hopkin 2005) make them susceptible to dehydration. Prolonged exposure to dry conditions causes negative physiological effects including reproductive failure, and, if long enough, death (Bayley and Holmstrop 1999). Tests of the effect of stressors on this species have revealed that individuals avoid dry or hazardous soils by migrating until they encounter a moist habitat (Fountain and Hopkin 2001; Hilligsøe and Holmstrop 2003; Krogh 2009). Although *F. candida* are capable of physiological adjustments to mitigate the effects of drought stress, these processes are metabolically costly (Bayley and Holmstrop 1999; Hilligsøe and

Holmstrop 2003) and require considerably more time than the habitat assessments (48 hours) I concentrate on here. The abundance of *F. candida* in highly contaminated soils declines through time (Fountain and Hopkin 2001), thus demonstrating that these Collembola appear capable of selecting safe habitat in response to environmental risk rather than persisting in bad conditions (Hiligsøe and Holmstrop 2003).

I reared animals according to ISO (1999) and Environment Canada (2007) standard protocols and maintained populations in sealed, transparent plastic chambers with a 1 cm thick substrate of 9:1 plaster of Paris (MSDS 00071008001) and activated charcoal (Laboratory grade, BIN:81255-03). I maintained laboratory cultures consisting of ~300 hatched animals in constant darkness at room temperature (mean = 21ºC; S.E = 0.5º C) and fed animals *ad libitum* yeast pellets (Fleischmann's® active dry yeast, *Saccharomyces cerevisae*) weekly. I saturated chambers with distilled water at feeding time and allowed them to aerate for five minutes.

I created new age-synchronized cultures for the risk-allocation experiments by introducing adults from multiple stock cultures into unoccupied growth chambers and allowed them to lay eggs on the smoothed substrate for 48 hours. Eggs found with the aid of a binocular dissection microscope (25-44× magnification) were carefully moved with wax-coated specimen pins to new chambers. Eggs hatched after seven to ten days. Juveniles were then allowed to grow to reproductive maturity (21-24 days old) while I renewed their food and re-moistened the chambers weekly.

Feeding treatment

I transferred a minimum of 30 Collembola from the synchronized rearing chambers into sterile 100 mm polyesterene disposable Petri dishes (Figure 3). I tilted the rearing chamber and carefully brushed adult individuals into each dish with a camel hair paintbrush. Counting animals at this stage was difficult, so the actual number of animals in some dishes was somewhat higher than the targeted density. Dishes were labelled according to replicate (N=10) and treatment (10). Treatments varied according to the proportion (p) of days during which food was absent, (p = 0, 0.25, 0.50, or 0.75) and duration *(d*, short, medium or long intervals of food and no-food conditions; Table 1). Controls (p = 0) were fed daily and were used to verify treatment effects on the mortality, reproduction and subsequent recruitment of offspring, not to test for differences in p or d on habitat selection. Five replicates were completed during winter 2014 (series one, 24 February to 3 April). Five additional replicates were completed in spring 2014 (series two, 19 March to 29 April).

I monitored all dishes daily and recorded the number of clutches and the number of living animals in order to gather information on the energetic state of individuals in the treatment populations. Eggs were removed daily to avoid altered energetic states caused by cannibalism and unwanted recruitment into the treatment populations. If animals escaped or perished on days one to seven, they were replaced using residual specimens from the synchronized stock population in order to minimize the difference in density between dishes. I assumed that the number of escaped individuals would be sufficiently small such that the introduction of new Collembola after day one would not alter a population's mean energetic state. After day seven, I counted the number of animals that

escaped or died, but did not introduce new animals that would have insufficient time to achieve the mean energetic state of the treatment population.

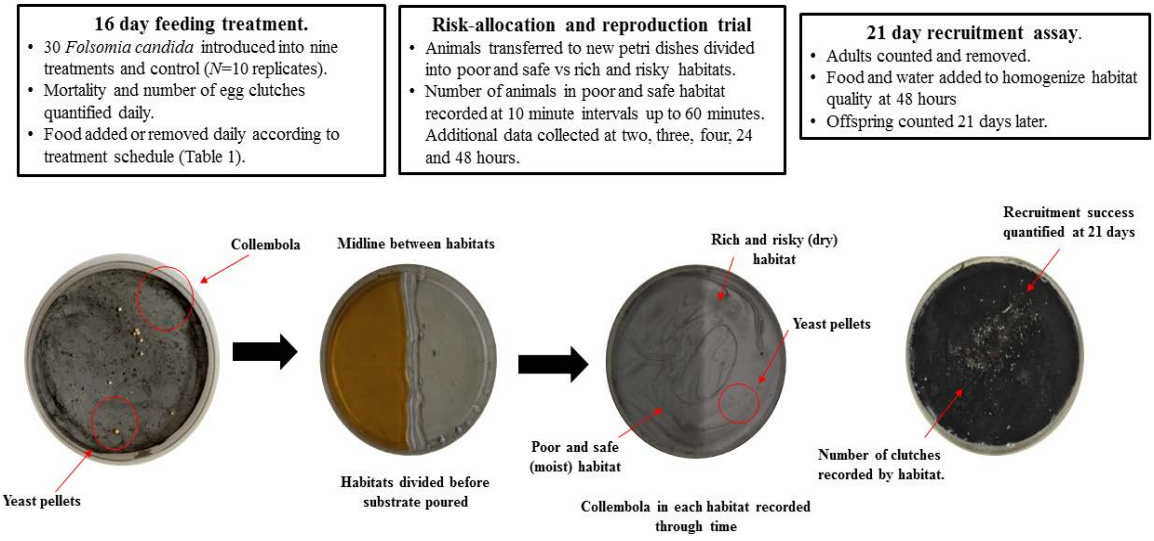

Figure 3: Top: Timeline for risk allocation experiments using the parthenogenic Collembolan *Folsomia candida.* Bottom: Photographs of petri dishes during the 16-day feeding treatments (left), examples of the moisture barrier and its effectiveness (middle), and a fully hydrated dish following risk allocation (right).

I added and removed food according to treatment (Table 1) and designed treatments based on the 24-hour gut turnover time of *F. candida* (Hopkin 1997). I maintained 100% humidity by pipetting 1000 µl of distilled water (100-1000 µl Mline® pipette) daily into the periphery of each dish. At the end of each feeding treatment I counted the surviving *F. candida* and the number of eggs (again using a binocular dissection microscope) to determine if there were significant differences in these fitness estimates among treatments.

Table 1: The 16-day sequence of food and no-food periods for each of nine feeding treatments and an always-food control. Values 0, 0.25, 0.50 and 0.75 correspond to the proportion of time that animals spent in no-food conditions; (*p*) indicates the relative duration (short, medium and long) of intervals with no food.

Day	**Treatment**									
	1 **0.25** **Short**	**2** **0.25** **Medium**	**3** **0.25** **Long**	**4** **0.50** **Short**	**5** **0.50** **Medium**	**6** **0.50** **Long**	**7** **0.75** **Short**	**8** **0.75** **Medium**	**9** **0.75** **Long**	**10** **0** **Control**
1	Food	Food	Food	Food	Food	Food	Food	Food	Food	Food
2	Food	Food	Food	No Food	Food	Food	No Food	Food	Food	Food
3	Food	Food	Food	Food	No Food	Food	No Food	No Food	Food	Food
4	No Food	Food	Food	No Food	No Food	Food	No Food	No Food	Food	Food
5	Food	Food	Food	Food	Food	No Food	Food	No Food	No Food	Food
6	Food	Food	Food	No Food	Food	No Food	No Food	No Food	No Food	Food
7	Food	No Food	Food	Food	No Food	No Food	No Food	No Food	No Food	Food
8	No Food	No Food	Food	No Food	No Food	No Food	No Food	No Food	No Food	Food
9	Food	Food	Food	Food	Food	Food	Food	Food	No Food	Food
10	Food	Food	Food	No Food	Food	Food	No Food	Food	No Food	Food
11	Food	Food	Food	Food	No Food	Food	No Food	No Food	No Food	Food
12	No Food	Food	Food	No Food	No Food	Food	No Food	No Food	No Food	Food
13	Food	Food	No Food	Food	Food	No Food	Food	No Food	No Food	Food
14	Food	Food	No Food	No Food	Food	No Food	No Food	No Food	No Food	Food
15	Food	No Food	No Food	Food	No Food	No Food	No Food	No Food	No Food	Food
16	No Food	No Food	No Food	No Food	No Food	No Food	No Food	No Food	No Food	Food

Risk-allocation trial

I transferred all *F. candida* in each dish at the end of the feeding treatments to a new petri dish where they could choose between two habitats of equal size: an arid food-rich habitat (with an overabundance of yeast pellets but risk of mortality through desiccation; hereafter rich and risky), and a moist habitat which lacked food (poor and safe). I created the two habitats by dividing the substrate into two equal parts with a 0.5 cm × 0.5 cm moisture barrier (Perma All-purpose all-temperature bonding material, ID 02-0200993). I then filled the habitat-selection petri dishes with the same plaster and charcoal substrate as in the feeding treatments and recorded the weight of each chamber.

I created the safe habitat by moistening one half of each dish with distilled water with a micropippetor in order to attain 50% saturation. A 50% saturation level yields 100% survival at 24 hours (Appendix 2). I created risky habitat by adding only enough distilled water to create 8% saturation. Some animals exposed to this saturation value began to suffer mortality at 60 minutes (Appendix 2).

I spread nine yeast pellets haphazardly throughout the rich and risky side, and none on the safe and poor side (*F. candida* can detect and travel to food sources at a distance of 25 mm, Auclerc *et al*. 2010). I then introduced the Collembola to the midline of each habitat-selection disk with a paper funnel and camel-hair paintbrush. I counted the number of animals occupying the poor and safe habitat at ten minute intervals to 60 minutes, then converted these values to proportions in order to accommodate differences in the total number of animals among dishes. *F. candida* are capable of fully exploring a petri dish within 10 minutes (Auclerc *et al*. 2010), so the one-hour trial presented

individuals with more than ample time to assess and select habitat according to their energetic state. Even so, I continued to count the number of individuals occupying the poor and safe habitat at 2, 3, 4, 24 and 48 hours after their introduction. Analyses of these data yielded qualitatively similar results to those collected during the first hour of observation (Appendix 2).

Reproduction and recruitment

I searched all dishes for egg masses at both the 24 hour and 48 hour intervals and recorded the number and location of clutches within each habitat. I used these correlates of fitness to quantify the relative energetic states of adults exposed to the different treatments (Crouau and Cazes 2003). I supplemented this test in the second set of replicates by discarding the adults at 48 hours. I added distilled water and yeast pellets to create a uniformly moist and rich substrate for juvenile Collembola, sealed the dishes with parafilm to maintain humidity, and counted all surviving offspring 21 days later using the floatation method described by Hopkin (1999). I photographed the Collembola within each dish using a Nikon D3200 digital camera, then displayed the images on a large computer monitor for counting. I used the number of offspring visible in these photos to gain an additional estimate of energetic state through the recruitment associated with each treatment.

Predictions

Mortality during feeding treatments

If *p* determines energetic state at the end of the experiment, and if energetic state influences survival, then *F. candida* should experience differential mortality among

feeding treatments; animals exposed to $p = 0.75$ should exhibit the highest mortality. Similarly, if duration (d), also determines energetic state, then mortality should increase with the length of no-food periods and especially so when $p = 0.75$.

Reproduction during feeding treatments

If animals in a high energetic state lay more eggs and p determines energetic state in *F. candida*, then mean fecundity should vary inversely with the proportion of time that animals existed without food. Additionally, if duration determines energetic condition, then animals exposed to rapid switching between environmental conditions would have higher fecundity than animals living in environments with long periods (with equal p) without food.

Mortality during the risk-allocation trial

Survival of *F. candida* during risk allocation should not vary significantly among feeding treatments if animals optimally select habitats during the 60 minute risk-allocation trial. However, if mortality is a consequence of previous energetic state, fewer *F. candida* should survive for 48 hours as either p or d increases.

Reproduction during the risk allocation trial

If energetic state determines fecundity and recruitment, then individuals exposed to rapid rates of switching or infrequent no-food conditions should produce more clutches and higher quality eggs during the risk-allocation trial. After the trial, these treatments would also have the highest offspring recruitment, and if animals in a high energetic state prefer the poor and safe habitat, then this habitat will contain more clutches and eggs than will the rich and risky habitat.

Habitat selection in the risk allocation trial

I designed the experiment with four different proportions of time without food. Thus, if p determines risk-allocation decisions in *F. candida*, then the rank of densities of Collembola within the poor and safe habitat, according to proportion and treatment (Table 1), should be:

$$(p = 0) > (p = 0.25) > (p = 0.5) > (p = 0.75);$$

$$10 > (1 = 2 = 3) > (4 = 5 = 6) > (7 = 8 = 9). \quad (3)$$

Conversely, if the risk allocation decisions are primarily influenced by the duration of no food periods, then the proportion of Collembola within poor and safe habitat according to duration and treatment (Table 1) should be:

$$0 \text{ days} > 1 \text{ day} > 2 \text{ days} > 3 \text{ days} > 4 \text{ days} > 6 \text{ days} > 12 \text{ days};$$

$$10 > (1 = 4) > (2 = 5) > 7 > (3 = 6) > 8 > 9. \quad (4)$$

Statistical analysis

Mortality and reproduction

I anticipated that fitness metrics (estimates of mortality and fecundity) within the feeding treatments and risk-allocation assay would not fit a normal distribution. Therefore, I used a set of Kruskal-Wallis H (hereafter, K-W) tests to determine if there were significant differences in these metrics among treatments and the control. K-W tests were appropriate as they resolve non-homogeneous variances that might not be remedied by transformations (Stam *et al*. 1996). I used pairwise comparisons to identify significant differences in 'fitness' caused by differences in the proportion and duration of no-food

periods. To assess significant differences in oviposition site during the risk-allocation trial, I used a one-tailed paired T-test to assess whether more eggs were laid in the safe habitat (where egg survival and juvenile recruitment should be highest) than in the risky one.

Habitat selection

I assessed habitat preference by Collembola with repeated measures Anolyses of Variance (ANOVA's, using dishes as subjects) to evaluate the between-subjects effects of series, treatment, proportion and duration on the proportion (arcsin transformed, McDonald 2014) of Collembola occupying the poor and safe habitat at 10 minute intervals (total of six counts). I used Kendall's tau to test which of the ranked predictions best matched the empirical pattern of habitat selection by *F. candida.* I used multiple ANOVAs to separately test for differences among series, treatments, and their interaction (treatments 1-10), proportion of time without food (treatments 1-9 pooled by proportion) and duration (treatments 1-6 pooled by one, two, or four days without food). Control data were not included in most analyses to prevent bias in the significant effect of either proportion or duration on habitat selection by *F. candida*. I used Tukey's HSD post-hoc analyses to identify which treatments, proportions and durations were significantly different from one another. For all repeated measures analyses where Mauchley's assumption of sphericity was violated ($P < 0.05$), I applied Greenhouse-Geisser corrections to alter the degrees of freedom and produce an F-ratio with a reduced Type I error rate (Greenhouse and Geisser 1959).

The ANOVAs revealed significant differences in habitat selection between the two series that used different batches of substrate and were separated in time. It is thus

possible that differences in substrate created differences in habitat-selection. I searched for this possibility by assessing differences between series in the mean weight of substrate, and volumes of distilled water, with a two-factor ANOVA.

All analyses were performed with IBM SPSS Statistics V.20. Raw data were entered and organized with Microsoft Excel 2010.

Results:

Mortality during feeding treatments

The number of surviving *F. candida* during the feeding treatments was high, with a mean mortality of only 5.8% (Table 2); the number of dead Collembola did not vary significantly among treatments and the control (K-W test, χ_9^2 = 3.55, *P* = 0.94, Table 3). The number of escaped Collembola over 16 days was higher with a mean value of 9.5%, but again, did not vary significantly among treatments and the control (K-W test, χ_9^2= 9.00, *P* = 0.44, Table 3). The mean density of surviving Collembola entering the risk allocation trial was 30.02 ± 0.79 individuals per dish.

Reproduction during feeding treatments

As predicted, well-fed *F. candida* (*p* = 0, 0.25) produced, on average, more clutches per day than did animals in other treatments (K-W test, χ_3^2 = 77.93, *P* < 0.001; Table 2 and Appendix 3). Control animals, and those that were fed for 75% of the feeding treatment (*p* = 0.25), produced the most clutches while individuals that were mostly food deprived tended to produce fewer clutches. Treatments six, seven and nine yielded fewer clutches per day than treatments one, two, three and ten. Treatment eight yielded fewer clutches than treatments one, two, three, four, five and ten (post-hoc pairwise comparisons; overall K-W test, χ_9^2 = 47.22, *P* <0.001, Table 3, Appendix 3). Despite differences in clutch numbers, there were no significant differences among treatments in the number of eggs laid (K-W test, χ_9^2 = 12.76, *P* = 0.17, Table 3).

Mortality during the risk allocation trial

Mortality during the risk-allocation trial was low. All animals survived through the 60 minute trial and only 183 of the 3,005 Collembola perished by 48 hours. More animals appeared to die in the poor and safe habitat (103) than in the rich and risky habitat (80), but the difference from expected values (assuming equal mortality) was only marginally significant (P = 0.09, Table 4).

Table 2: Summary of mortality, escape, clutch production and mean fecundity of *F. candida* during a 16-day feeding trial.

Treatment	**% Mortality**	**% Escape**	**Mean number of clutches per day (*N*=10)**	**Mean number of eggs (day 17; *N*=10)**	**Mean number of survivors (*N*=10)**
1. 25%, short	6.95	3.64	2.59	269.6	30.2
2. 25%, med.	4.36	8.72	2.70	172.2	29.8
3. 25%, long	5.0	6.33	2.52	50.2	30.0
4. 50%, short	5.67	10.33	2.08	26.06	30.0
5. 50%, med.	7.62	5.96	2.13	79.2	30.2
6. 50%, long	3.01	8.36	1.53	79.2	29.9
7. 75%, short	4.38	15.15	1.59	156.8	29.7
8. 75%, med.	8.39	7.05	1.20	32.4	29.8
9. 75%, long	5.65	19.93	1.44	45.2	30.1
10. Control	6.56	9.84	2.95	195.0	30.5
Mean ± S.D	5.76 ± 1.56	9.53 ± 4.54	2.08	120.59 ± 78.42	30.02 ± 0.23

Reproduction during the risk allocation trial

The vast majority of clutches (482 of 492) was located in poor and safe habitat (Paired T-test: $T_9 = 8.15$, $P < 0.001$). The mean number of clutches laid varied significantly among treatments and the control (K-W Test, $\chi^2_9 = 38.84$, $P < 0.001$, Table 5 and Appendix 3). Treatment nine produced significantly fewer clutches than treatments one, two, three, five, and six (post-hoc pairwise comparisons).

Offspring recruitment

Well-fed Collembola, as predicted, produced more recruits than Collembola starved for $p = 0.75$ of the time (K-W test, $\chi^2_3 = 21.08$, $P < 0.001$, Table 3). Treatments eight and nine yielded fewer recruits than treatment five (and nine less than two; post-hoc pairwise comparisons; overall K-W test: $\chi^2_9 = 28.48$, $P < 0.001$, Figure 4, Table 3).

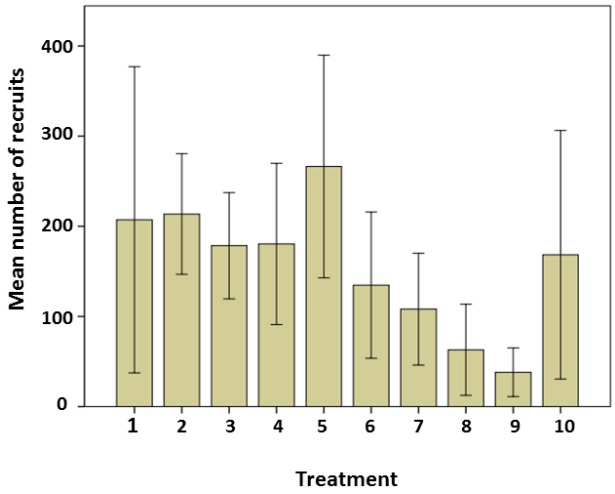

Figure 4: Mean (± *SE*) recruitment of *F. candida* among feeding treatments.

Table 3: Summary of the single-classification contingency analysis evaluating the number of dead Collembola in safe versus risky habitat at 48 hours in the risk-allocation trial.

	Observed	Expected
Number of dead Collembola (Safe habitat)	103	91.5
Number of dead Collembola (Risky Habitat)	80	91.5

$\chi_1^2 = 2.89$, $P = 0.09$

Table 4: Summary of Kruskal-Wallis tests evaluating a) mortality and escape during the 16-day feeding treatment b) reproduction during the feeding treatments, number of clutches and number of eggs on day 17 c) reproduction during the risk allocation trial, and d) offspring survival until adult age; analyses by treatment and proportion (21 days).

Analysis*	***df***	χ^2	***P***
a) Mortality ($N = 100$)	9	3.55	0.94
Escape ($N = 100$)	9	9.0	0.44
b) Number of clutches ($N = 100$)	9	47.22	< 0.001
Number of eggs ($N = 100$)	9	12.76	0.174
c) Number of clutches ($N = 100$)	9	38.84	< 0.001
d) Recruits ($N = 100$)	9	28.48	< 0.001
Recruits by proportion ($N = 100$)	3	21.08	< 0.001

* Proportion included as a nominal variable.

Table 5: Summary of mortality and clutch production at 48 h, and selection of safe habitat at one hour, by *F. candida.*

Treatment	Mean # of clutches (*N*=10)	Mean # of dead animals (*N*=10)	Proportion in safe habitat at one hour (Series 1)	Proportion in safe habitat at one hour (Series 2)
1. 25%, short	5.5	0.5	0.61	0.60
2. 25%, med.	7.2	0.9	0.69	0.61
3. 25%, long	6.3	0.1	0.58	0.50
4. 50%, short	3.9	1.1	0.62	0.51
5. 50%, med.	11.0	0.5	0.65	0.47
6. 50%, long	5.6	1.2	0.58	0.50
7. 75%, short	2.4	0.7	0.42	0.45
8. 75%, med.	1.6	6.5	0.54	0.38
9. 75%, long	0.4	3.6	0.30	0.36
10. Control	4.4	3.3	0.83	0.48
Grand mean ± S.D	4.83 ± 5.6	1.84 ± 5.86	0.58 ± 0.18	0.49 ± 0.14

Habitat selection

Habitat selection by Collembola varied significantly among treatments (rmANOVA: $F_9 = 14.14$, $P < 0.001$, Table 6). The ranking of relative Collembolan density in the safe habitat by treatment was

$$10 > 2 > 1 > 4 = 5 > 3 = 6 > 7 = 8 = 9.$$

This order of relative densities supports the prediction that *F. candida* risk-allocation behaviour was more likely determined by *p* than by the duration of time without food (rank correlation with inequality (3), $\tau = 0.87$, $P < 0.001$; rank correlation with inequality (4), $\tau = 0.64$, $P = 0.009$).

Further analysis confirmed the significant effect of *p* on habitat selection by *F. candida* during the one-hour trials of risk-allocation behaviour (rmANOVA: $F_{2, 87} = 20.30$, $P < 0.001$, Table 6). The relative densities of Collembola within safe habitat were substantially lower for animals starved 75% of the time compared with those starved for 0, 25 and 50% of the time (Tukey's HSD $P < 0.001$; Figure 5A).

In contrast, the main effect of duration did not significantly influence the habitat choice of *F. candida* for treatments one to six (rmANOVA: $F_{2, 57} = 0.95$, $P = 0.39$, Table 6) nor did it have a significant effect on the risk allocation by *F. candida* among treatments seven, eight, or nine (rmANOVA, $F_{2, 27} = 2.26$, $P = 0.12$, Table 6). Control populations maintained higher relative densities within safe habitat than populations exposed to no-food conditions (Tukey's HSD, $P < 0.001$).

The relative density of animals within the poor and safe habitat also varied between series (rmANOVA: $F_{80}^{1} = 15.85$, $P < 0.01$, Tables 5 and 6), suggesting a significant substrate effect. Substrate weight was indeed greater in series two (14.4 ± 0.97 g) than in series one (11.74 ± 2.41 g; $F_{98}^{1} = 97.99$, $P < 0.001$, Table 6), even though the recipe was identical. It thus appears that the moisture content of substrate was higher in series two than it was in series one. However, the ability to detect significant habitat selection in spite of such variation indicates a reliable test of risk-allocation theory.

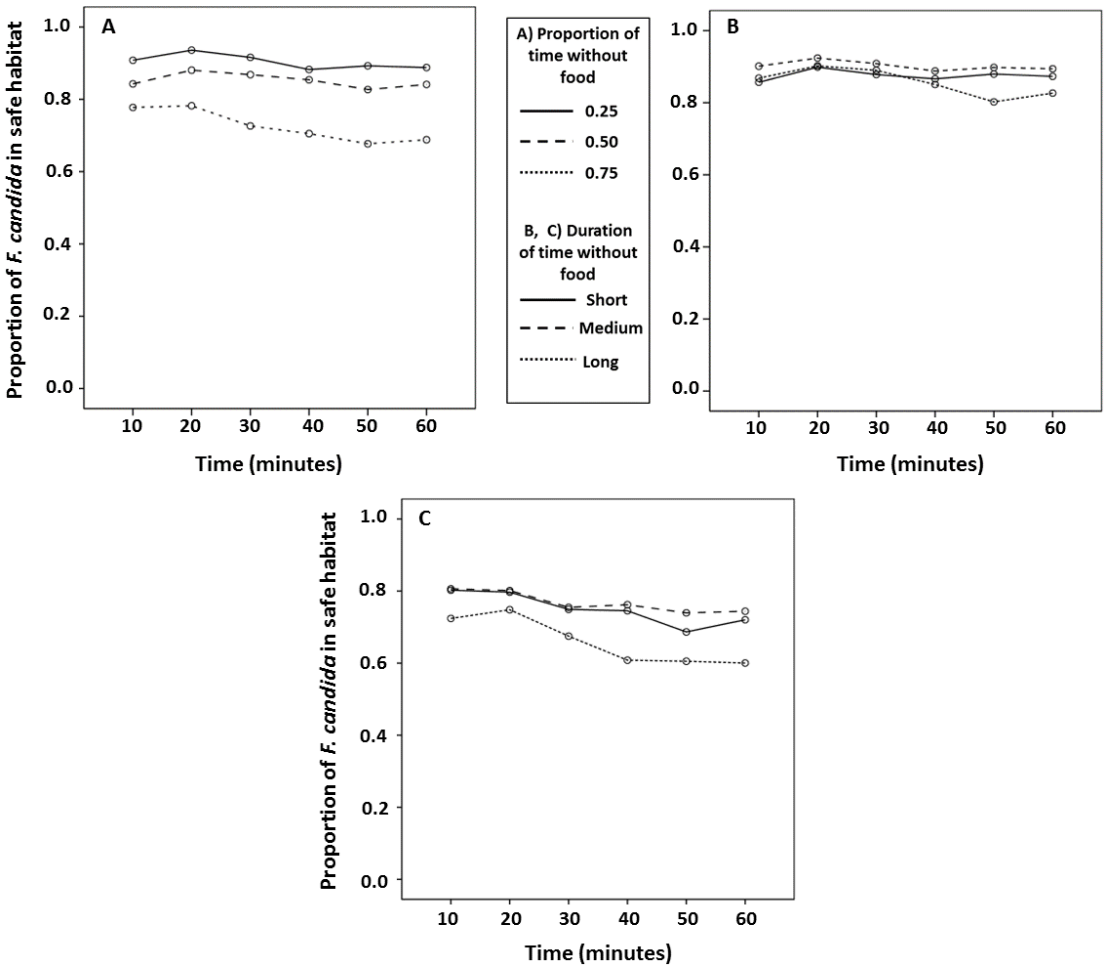

Figure 5: Occupation of poor and safe habitat during risk allocation by *Folsomia candida*. Panel A displays the mean proportion of Collembola occupying the poor and safe habitat at 10, 20, 30, 40, 50 and 60 minutes based on the proportion of days without food; Panels B and C display the proportion of Collembola occupying the poor and safe habitat through time for populations experiencing different durations of days without food (B: short = 1 day, medium = 2 days, long = 4 days; treatments one to six only; C: short = 3 days, medium = 6 days, long = 12 days, treatments seven to nine only).

Table 6: Summary of significant results from four models evaluating habitat selection by food-deprived *F. candida* *.

Analysis and source	***df***	***F***	***P***
1. rmANOVA (Treatment):			
Treatment	9	14.14	< 0.001
Series	1	15.85	< 0.001
Error	89		
2. rmANOVA (Proportion):			
Proportion	2	20.30	< 0.001
Error	87		
3. rmANOVA (Duration):			
Treatments 1-6:			
Duration	2	0.95	0.39
Error	57		
Treatments 7, 8 and 9:			
Duration	2	2.26	0.12
Error	27		
4. Two-Way ANOVA:			
Substrate Weight	1	97.99	< 0.001
Error	98		

*Series: nominal variable (1=series 1, 2 = series 2) coding for the temporal series of treatment replicates. Proportion: ordinal variable (1 = 0.25, 2 = 0.50, 3 = 0.75, 4 = 0) coding for the proportion of time that a feeding treatment spent with no food. Treatment: nominal variable (1 = treatment 1, 2 = treatment 2, 3 = treatment 3, 4 = treatment 4, 5 = treatment 5, 6 = treatment 6, 7 = treatment 7, 8 = treatment 8, 9 = treatment 9, and 10 = Control). Substrate weight: continuous variable of substrate dry weight (*g*) in each petri dish used in the risk-allocation trial.

Discussion

Risk-allocation decisions are a form of optimal foraging behaviour that depend on the broader context in which environmental risk varies (Lima and Bednekoff 1999; Sih and McCarthy 2002). Such decisions can determine whether an animal survives in a stochastic environment or succumbs to either starvation or predation within a given time interval (Brown *et al*. 1999; Higginson *et al*. 2012). My experiments with *F. candida* are consistent with previous studies documenting that the risk-allocation behaviour of foragers emerges through temporal patterns of environmental change that influence energetic state (Ferrari et al. 2009; Sih and McCarthy 2002).

Exposing *F. candida* to different feeding treatments forced them into a variety of energetic states that subsequently influenced their choice of a rich habitat with desiccation stress over a poor, moist one (Figure 6). Populations that had frequent and regular access to food selected safe habitat more often, while animals deprived of food for 75% of the time selected the rich and risky habitat despite risk of mortality through desiccation.

Most importantly, two surrogates of fitness (the number of clutches and number of recruits) confirmed my assumption that Collembola exposed to different feeding treatments emerged in different energetic states. Well-fed *F. candida* produced more clutches and recruits than poorly-fed individuals. Animals in a high energetic state were thus more selective in oviposition site, and likely produced higher quality eggs, than individuals in a lower energetic state. These results are consistent with the interpretation that reproductive success should reflect changes in environmental condition (Ludwig and

Rowe 1990), and especially so in *F. candida* (Staempfli *et al*.2007; Tully and Ferriere 2008). It is thus reasonable to assume that differential habitat selection in my experiments was a consequence of adaptive risk allocation in response to depleted energy reserves.

The disparity in energetic condition between Collembola exposed to 25% versus 75% no-food conditions suggests the presence of an energetic threshold (Brown *et al*. 1997), below which animals must forage in rich and risky habitat (Figure 6). This conclusion is supported by my fitness surrogates. Animals fed more frequently had higher fecundity than those fed for only 25% of the time. Oddly, differences among treatments in the number of clutches were not mirrored by similar differences in the total number of eggs. These anomalies can be attributed to reproductive asynchrony among feeding populations. My synchronized populations were created by accumulating eggs between 0-48 hours old, causing slight differences in age (or reproductive phase, Appendix 1) among treatment populations (Crouau and Cazes 2008). Regardless, the significant influence of both treatment and *p* on offspring recruitment and clutch number is consistent with the predictions of risk allocation. These data indicate that state dependent habitat selection as a surrogate of risk allocation appropriately tracks fitness differences across varying environmental conditions (Morris and Davidson 2000).

My results dispute the claim that the predictions of the original risk-allocation hypothesis are inaccurate (Beauchamp and Ruxton 2011; Higginson et al. 2012). Inconsistencies in tests of risk-allocation theory are unlikely caused by shortcomings of the hypothesis itself, but are the result of a poor fit between test conditions and the theory's key assumptions (Ferrari *et al*. 2009; Higginson *et al*.2012; Lima and Bednekoff

2011). Tests of risk allocation require careful and appropriate experimental design specific to the system and organism to which they are applied.

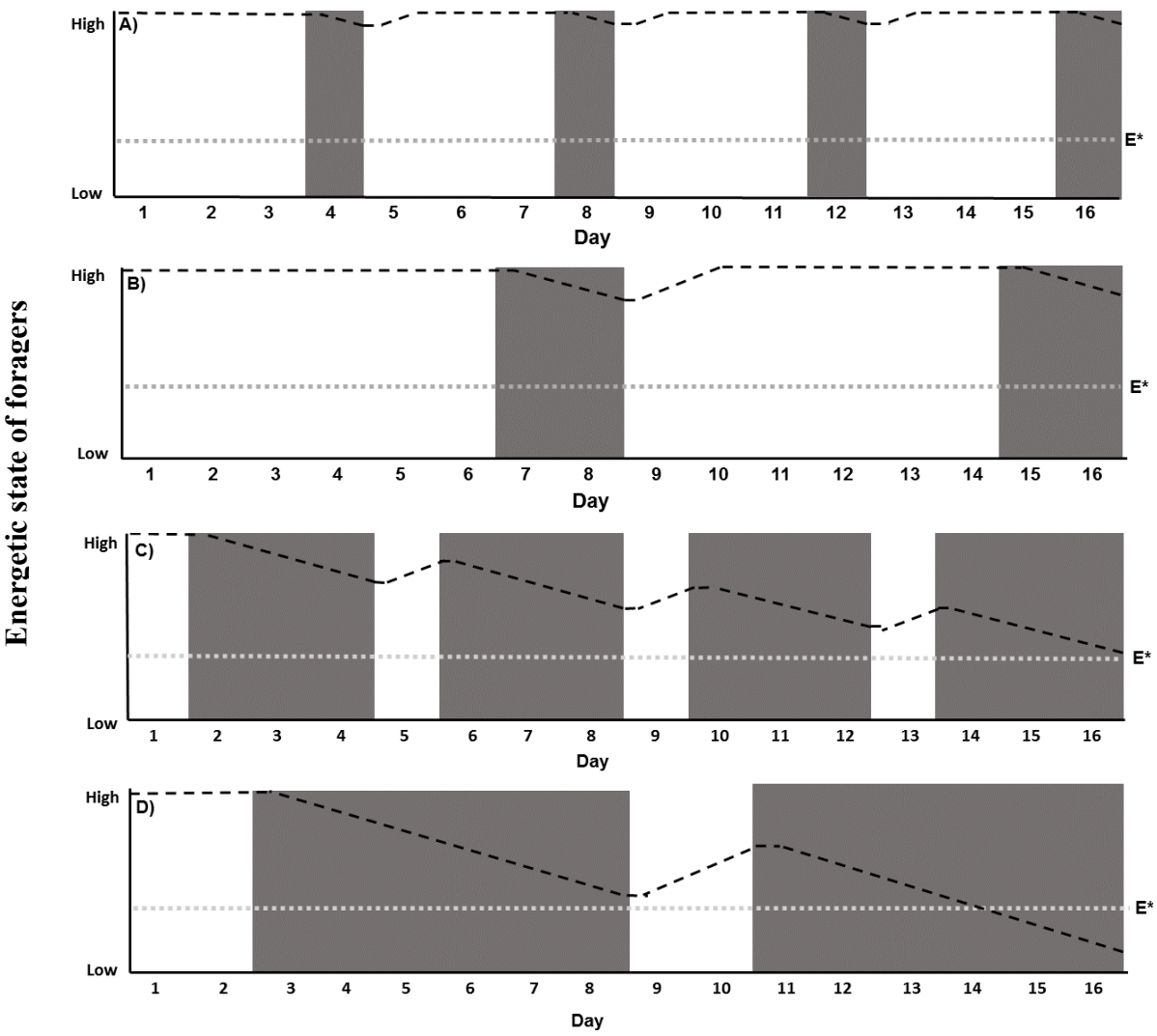

Figure 6: A caricature of the energetic state in *F. candida* exposed to four different sequences of food and no-food (shaded bars) conditions within 16-day feeding treatments. The energetic state of foragers (low to high) is represented by the dashed line in each panel. Individuals are assumed to acquire and dispose of resources at an equal rate. Panel A represents a treatment ($p = 0.25$, short duration) in which Collembola were easily able to meet their energetic requirements E^* (dotted grey line) before the end of the trial. Panel B ($p = 0.25$, medium duration) represents a treatment with elongated periods without food but individuals still met their energetic requirements. Panels C and D illustrate two treatments for which the environment was dominated by no-food conditions ($p = 0.75$). In these treatments, the short periods of foraging exceeded the foragers' ability to restore energy reserves and individuals declined to low energetic states. In Panel D, individuals risked starvation if they did not feed immediately following the end of the treatment.

The inclusion of temporal autocorrelation in environmental conditions nevertheless reveals the importance of a predictable pattern of resource acquisition in order to accurately forecast risk allocation behaviour from the energetic state of foragers (Higginson et al. 2012, Figure 6). Ensuring the proper manipulation of energetic condition within test organisms, and allowing foragers sufficient time to adjust to environmental risk regimes, are also imperatives to proper tests of the theory (Sundell *et al.* 2004; Vainekka *et al.* 2005). Animals that are not physiologically stressed do not adhere to the principles of the risk allocation model, while organisms that are not pre-exposed to patterns of environmental risk may be unable to allocate risk accordingly (Ferrari *et al*. 2009).

Two *caveats* apply to my tests of risk allocation. First, my test is complicated by the difficulty of designing experiments where the environmental effects of proportion and duration of time spent without food, are independent of one another. In tests of risk-allocation theory with *F. candida*, habitat selection corresponds with the prediction that the proportion of time that I exposed animals to risk determines risk-allocation behaviour. Second, without data on individual habitat selection, body weight, lipid and protein content, I cannot definitively conclude that subtle changes in energy reserves did not occur among individuals, and that these differences were not reflected in their habitat choices (Staempfli *et al.* 2007). Although this second caveat is important when assessing individual decisions, habitat selection is best viewed as a population response that varies with density and the frequency of alternative strategies (Morris 2011).

Logistical issues further confuse interpretation of the data. I was initially surprised that the proportion of animals in the safe habitat was higher in series one than in series

two. I attribute the difference in habitat preference to differences in substrate weight. The higher mean 'dry weight' in series two suggests that I did not attain total evaporation of water in the plaster of Paris (substrate was air-dried and changes in relative humidity necessarily affect moisture concentration). Although differential water saturation would have no appreciable effect on the safety of the poor habitat, it would reduce desiccation risk in the rich one. The important point is, however, that even though the relative abundance of *F. candida* within the safe habitat varied, general patterns of habitat selection remained consistent between series. Populations deprived of food most frequently were in a lower energetic state and preferentially occupied the rich and risky habitat relative to populations receiving food more frequently.

The demonstrated differences in habitat choice among populations in putatively different mean energetic states adds to the growing evidence that habitat selection reflects innate habitat quality (Knight *et al*. 2008; Olsson et al. 2002). Foraging behaviours provide insight into the condition of individuals, habitats and communities (Kotler *et al*.2007). Through an intrinsic link with fitness (individuals require energy to survive and reproduce), we can utilize repeatable foraging behaviours varying in response to environmental stochasticity to detect changes in environmental condition and quality. Monitoring changes in risk-allocation behaviour can thus inform us of changes in the foraging profitability of different habitats (Kotler *et al*. 2007; Morris and Davidson 2000) and allow wildlife and conservation managers to counteract habitat change before the abundance and distribution of individuals and populations are irrevocably altered (Kotler *et al*. 2007).

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Appendix 1: Life history of *Folsomia candida* (Willem)

Folsomia candida populations are composed of primarily parthenogenic females (males occur with a frequency of less than one in 1000 animals, Fountain and Hopkin 2005; Krogh 2009; OECD 2009). Reproduction in *F. candida* is believed to occur through the presence of *Wolbachia* (bacterium) in the ovarian cells, fat bodies and interstitia of females, but the mechanics of this process are not yet fully understood (Krogh 2009).

F. candida become reproductively mature 21 to 24 days after hatching, at the sixth (adult) instar. Adult lifespan ranges from 111 days at 24^{o}C to 240 days at 15^{o}C (Fountain and Hopkin 2005; Krogh 2009). Females can pass through as many as 45 instars in their lives, with each comprised of eight and a half non-reproductive days followed by 36 hours of reproductive capability (Fountain and Hopkin 2005). Females oviposit 30-50 eggs into a communal clutch during each reproductive phase. Many of the life history processes of *F. candida* depend on temperature, with colder temperatures prolonging life history stages and temperatures exceeding 28^{o}C resulting in reproductive failure. The average number of eggs laid by a female in her lifetime spans between 1100, 900 and 100 eggs at 15, 21 and 27^{o} C respectively (Hopkin 1997).

Each instar is followed by a moult in which the skin and mid-gut lining are shed (Krogh 200). Gut turnover time of *F. candida* is approximately 24 hours (Kaersgaard et al. 2004) with waste and ingested toxins stored in the midgut. Waste is voided into the lumen and is excreted as faeces during moulting (Fountain and Hopkin 2005).

Adult *F. candida* are between 1.5- 3 mm in length and are soft-bodied lacking external photoreceptors (Fountain and Hopkin 2001, 2005; Fox *et al.* 2007). The

integument of *F. candida* is highly permeable with oxygen uptake occurring through the cuticle and fluid exchange with the external environment made possible by a Collophore (paired eversible vesicles within the abdominal segment, Fountain and Hopkin 2005).

F. candida individuals are eudaphic and are rarely exposed to relative humidity lower than 96% (Kaersgard *et al*. 2004). *F. candida* employs both behavioural and physiological adaptations to combat desiccation stress. During acute exposure to dry soil conditions, adult *F. candida* are able to migrate both horizontally and vertically within the soil column as well as readily absorb water vapour to remain active below 98.9% relative humidity (Fountain and Hopkin 2005). If the risk of dehydration persists, adult *F. candida* begin to increase the osmolality of their haemolymph by synthesizing myoinositol in order to re-establish hyperosmoticity (Fountain and Hopkin 2005; Kaersgaard *et al*. 2004). The increased production of cryoprotectants (glucose and myoinosital) confers cellular protection against chronic drought and cold stress (Kaersgaard et al. 2004). At dehydration levels below 90% relative humidity, or in incidences of acute, severe dehydration exposure, *F. candida* relies exclusively on behavioural methods, such as migration, to avoid mortality (Bayley and Holmstrop 1999).

Appendix 2: Substrate saturation index

In order to create high and low quality habitats within the risk allocation chamber, I needed to determine the correct amount of moisture for each habitat. Proper moisture levels were especially critical in the rich and risky habitat which was intended to impose physiological stress on Collembola without causing significant mortality over the course of the hour-long risk allocation assessment.

To obtain reference values for moisture saturation, I added 30 Collembola to 42 dishes suffused with distilled water. The amount of water added to the substrate of each dish varied from 0-100% saturation, where 100% saturation was established by taking the volume of water (11.73 ml) required to generate barely visible surface water on substrate of a known mean dry weight (11.735 ± 2.407 SD). After the first round of testing, the saturation value for slow mortality was deemed to be 8% or greater (one animal died after 40 minutes, all were dead at 24h, Table 2.1). All animals exposed to 10% saturation survived, so I tested survival in the range between 8.2% and 10% (Table 2.2) to identify the threshold for Collembola mortality over 24 hours. Results at this finer scale of saturation confirmed the animals occupying arid habitat up to 8.4% saturation faced increased risk of mortality through time (Table 2.3). For the poor and safe habitat, 50% saturation was selected as the optimum moisture level that provided tolerable conditions without increasing the likelihood that water from the moist habitat would increase the water content of the dry one.

In order to most accurately create 8% and 50% saturation levels in risk allocation dishes, 65 dishes were created for each series. Each dish was weighed before adding

liquid substrate then allowed to air dry for 72 hours. Care was taken to ensure that the initial substrate slurry was of uniform depth on both sides of the moisture barrier. Each dish was then re-weighed in order to calculate the dry weight of substrate in each dish, and the deviation from the mean of all dishes. Data for the 15 dishes that deviated most from the mean dry weight were discarded. The dry weight was then used to adjust the volume of distilled water required to create the safe (50% saturation) and risky (8% saturation) habitat of every dish. The corrected volume of distilled water was divided by two in order to obtain the required moisture for one half of the petri dish (Table 2.3). For example, assuming that 1 ml of water = 1 g, I added 0.534 ml of distilled water on the 8% side of a dish with 13.36 g substrate, and 3.34 ml of distilled water to the 50% side of the dish.

I added the requisite volume of distilled water with a micropippetor and left the dishes undisturbed for five minutes in order to allow the water to homogenize within the substrate. Collembola were introduced to the dishes and their mortality and number of clutches recorded up to 24 hours (Table 2.2).

Duration of risk allocation trial

Tests of risk allocation in *F. candida* were conducted at three different timescales. Primary testing occurred at 10-minute intervals up to one hour. In order to confirm that patterns of habitat selection in Collembola were consistent through time, data were also collected at two, three and four hours. These data were qualitatively similar with those collected during the one hour trial (Table 2.3).

Table 2.1: Summary of the results of mortality tests on 33 populations of *F. candida* up to 24 hours. Collembola were exposed to substrate between 0-100% saturation (ml) with distilled water.

		Mortality (number of dead animals)								
Saturation (%)	**Water (ml)**	**20 minutes**	**40 minutes**	**60 minutes**	**120 minutes**	**180 minutes**	**240 minutes**	**300 minutes**	**360 minutes**	**24 hours**
0	0.000	30	30	30	30	30	30	30	30	30
0.2	0.023	12	30	30	30	30	30	30	30	30
0.4	0.047	5	12	30	30	30	30	30	30	30
0.6	0.070	3	5	6	30	30	30	30	30	30
0.8	0.094	5	13	15	30	30	30	30	30	30
1	0.117	4	10	11	19	30	30	30	30	30
1.2	0.141	7	11	12	13	18	30	30	30	30
1.4	0.164	4	5	5	9	12	21	30	30	30
1.6	0.188	3	4	5	5	5	5	30	30	30
1.8	0.211	8	8	8	8	9	9	29	30	30
2	0.230	9	9	12	15	15	15	30	30	30
4	0.469	11	12	12	12	14	14	14	14	30
5	0.570	1	1	2	4	4	4	4	4	30
6	0.704	2	5	5	5	6	6	6	6	30
8	0.938	0	0	1	1	1	1	1	1	30
10	1.170	0	0	0	0	0	0	0	0	0
12	1.408	0	0	0	0	0	0	0	0	0
14	1.642	0	0	0	0	0	0	0	0	0
15	1.760	0	0	0	0	0	0	0	0	0
16	1.877	0	0	0	0	0	0	0	0	0
18	2.111	0	0	0	0	0	0	0	0	0
20	2.350	0	1	1	1	1	1	1	1	1
22	2.581	0	0	0	0	0	0	0	0	0
22	2.581	0	0	0	0	0	0	0	0	0
24	2.815	0	0	0	0	0	0	0	0	0
25	2.930	0	1	1	1	1	2	2	2	2
30	3.520	0	0	0	0	0	0	0	0	0
35	4.110	0	0	0	0	1	1	1	1	1
40	4.690	0	0	0	0	0	0	0	0	0
44	5.280	0	0	0	0	0	0	0	0	0
50	5.870	0	0	0	0	0	0	0	0	0
75	8.798	0	0	0	0	0	0	0	0	0
100	11.73	0	0	0	0	0	0	0	0	0

Table2.2: Mortality of *F. candida* living in habitats between 8 – 10% substrate moisture in order to identify desication tolerance.

		Mortality (number of dead animals)								
Saturation (%)	**Water (ml)**	**20 minutes**	**40 minutes**	**60 minutes**	**120 minutes**	**180 minutes**	**240 minutes**	**300 minutes**	**360 minutes**	**24 hours**
8.2	0.960	0	0	0	0	0	1	1	1	30
8.4	0.985	0	0	0	0	0	0	0	0	30
8.6	1.010	0	0	0	0	0	0	0	0	1
8.8	1.030	0	0	0	0	0	0	0	0	0
9.0	1.060	0	0	0	0	0	0	0	0	0
9.2	1.070	0	1	1	1	1	1	1	1	1
9.4	1.100	0	0	0	0	0	0	1	1	1
9.6	1.130	0	0	0	0	0	0	0	0	1
9.8	1.150	0	0	0	0	0	0	0	0	0

Table 2.3: Mean ($\bar{X}$± standard error) densities of *F. candida* within poor and safe habitat at nine time steps during the risk allocation trial.

	Time (minutes)																	
	10		20		30		40		50		60		120		180		240	
Treatment	$\bar{X}$	±SE	$\bar{X}$	±SE	$\bar{X}$	±SE	$\bar{X}$	±SE	$\bar{X}$	±SE	$\bar{X}$	±SE	$\bar{X}$	±SE	$\bar{X}$	±SE	$\bar{X}$	±SE
1	17.90	1.239	19.10	1.464	18.70	1.506	17.30	1.613	18.10	1.394	18.30	1.30	16.90	1.140	15.400	1.204	16.70	1.136
2	20.50	0.898	20.50	0.792	19.90	0.605	19.20	0.757	19.80	1.052	19.30	0.978	17.50	1.319	17.40	1.416	17.30	1.116
3	17.30	1.752	18.30	1.367	17.70	0.920	16.90	0.690	16.30	1.033	16.20	1.254	14.70	1.476	14.80	1.083	12.50	1.344
4	16.40	1.40	17.50	0.847	16.70	1.106	17.40	0.872	17.40	1.137	16.80	1.093	15.50	1.803	14.0	0.907	13.50	1.046
5	16.10	0.912	17.40	1.166	17.20	0.987	16.70	0.967	16.600	0.968	16.90	1.130	15.10	1.080	14.80	1.162	14.70	1.795
6	17.60	0.846	18.40	0.945	18.30	0.932	16.90	0.90	14.60	0.819	16.10	0.924	13.50	1.138	13.40	1.507	13.90	1.299
7	15.30	1.491	15.20	1.181	13.80	1.323	13.70	1.342	12.10	1.449	13.0	1.183	11.40	1.118	10.0	0.989	9.10	0.948
8	15.50	1.003	15.20	1.504	14.0	1.265	14.20	1.340	13.60	1.477	13.70	1.309	11.90	1.810	12.0	1.619	10.30	1.065
9	13.20	1.162	14.0	1.308	12.0	1.751	10.20	1.467	10.10	1.595	10.0	1.571	7.800	1.576	8.30	1.984	7.40	1.708
10	21.30	1.995	19.20	2.691	19.20	2.516	19.80	2.380	20.40	2.561	19.90	2.514	18.80	2.724	17.40	2.954	19.40	2.320

Appendix 3: Boxplots associated with K-W tests of clutch number

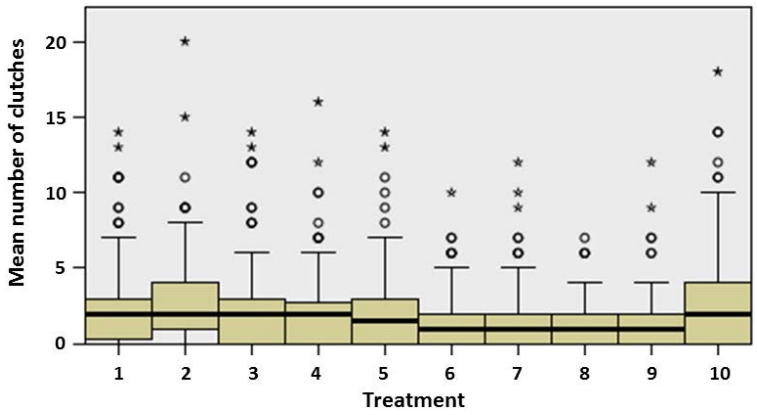

Figure 3.1: The mean number of clutches produced daily by nine treatments and a control (10) during a 16-day feeding treatment. Bars and boxes represent the median and interquartile (IQR) range respectively, whiskers extend to 1.5 × IQR, circles to outliers between 1.5 × IQR, and 3× IQR, and asterisks to outliers beyond 3× IQR.

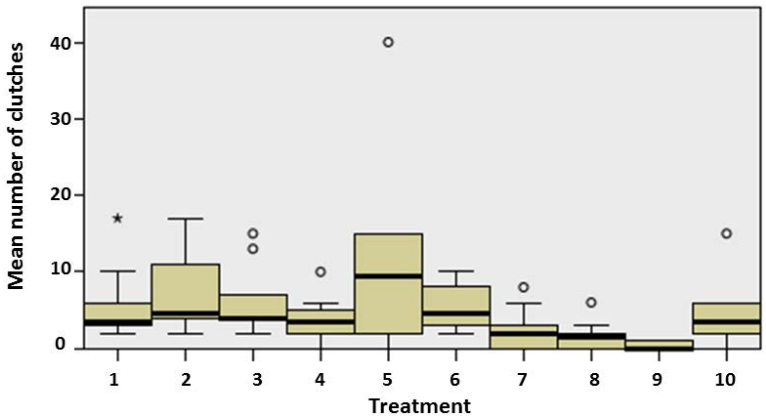

Figure 3.2: The mean number of clutches produced daily by nine treatments and a control (10) during the 48-h risk allocation (habitat selection) trial. Bars and boxes represent the median and interquartile (IQR) range respectively, whiskers extend to 1.5 × IQR, circles to outliers between 1.5 × IQR, and 3× IQR, and asterisks to outliers beyond 3× IQR.