Can temperature modify the strength of density-dependent habitat selection in ectotherms? A test with red flour beetles

W. D. Halliday & G. Blouin-Demers

Department of Biology, University of Ottawa, Ottawa, ON, Canada

Abstract

Habitat selection is an important aspect of the ecology of animals and models predict that density dependence is a strong force shaping patterns of habitat selection. In ectotherms, however, density dependence of fitness tends to weaken as temperature deviates from the species' optimal temperature (T_o). This may have important implications for density-dependent habitat selection because the underlying mechanism for density-dependent habitat selection is density dependence in fitness. We examine how temperature can modify the predictions from isodar theory and obtain temperature-dependent predictions for density-dependent habitat selection. We specifically predict that the isodar's intercept will be furthest from zero and the slope will be steepest at the optimal temperature. As temperature deviates from the optimal temperature, we predict that the intercept will approach zero and the slope will approach one. We then test these predictions with experiments on habitat selection based on food abundance by red flour beetles in the laboratory. We also confirm that fitness decreases as density increases and that density dependence weakens as temperature deviates from T_o. In agreement with our predictions, preference for habitats with more food weakened as density dependence weakened. Our results have implications for habitat selection by ectotherms because we demonstrate that variation in environmental temperature can weaken markedly the effect of density on both fitness and habitat selection. High density may entail no fitness costs for ectotherms that cannot maintain their optimal temperature.

Introduction

The distribution and abundance of organisms between habitats is a fundamental aspect of ecology (Fretwell & Lucas, 1969; Rosenzweig, 1981; Morris, 2003; Morris, 2011; Buckley, Hurlbert & Jetz, 2012). Habitat selection models attempt to explain patterns in animal distribution and abundance by relating habitat suitability to fitness (Fretwell & Lucas, 1969; Morris, 2011). In the simplest sense, each habitat represents a set of resources that differ from the sets in other habitats and the set of resources dictates the fitness achieved in each habitat (Fretwell & Lucas, 1969). Animals maximize fitness by choosing to live in the habitat offering the greatest fitness rewards and the mean fitness in each habitat decreases as population density increases due to increased competition (Fretwell & Lucas, 1969). Under the ideal-free distribution (IFD; Fretwell & Lucas, 1969), individuals in a population maximize fitness via their density-dependent distribution between habitats such that mean fitness is the same in all habitats, but decreases as density increases. The IFD has three main assumptions: (1) individuals have equal competitive abilities, (2) individuals have ideal knowledge of the distribution and suitability of habitats and of the distribution of competitors between those habitats and (3) individuals are free to move between habitats without travel costs (Fretwell & Lucas, 1969). Despite the unrealistic assumptions of the IFD (Kennedy & Gray, 1993; but see Åström, 1994 and Milinski, 1994), density-dependent habitat selection has been demonstrated in a variety of organisms, from endothermic vertebrates such as birds (e.g. Jensen & Cully, 2005; Zimmerman *et al.*, 2009) and mammals (e.g. Rosenzweig & Abramsky, 1986; Morris, 1988; Morris, Dupuch & Halliday, 2012), to ectothermic vertebrates such as lizards (Calsbeek & Sinervo, 2002) and fish (e.g. Rodríguez, 1995; Haugen *et al.*, 2006; Knight, Morris & Haedrich, 2008), to invertebrates (Krasnov, Khokhlova & Shenbrot, 2003; Krasnov *et al.*, 2004; Lerner *et al.*, 2011; Halliday & Blouin-Demers, 2014; Halliday & Blouin-Demers, 2016a).

Environmental temperature is a major component of habitat suitability for ectotherms due to the powerful effects temperature exerts on all aspects of their biology (Huey, 1991; Blouin-

Demers & Weatherhead, 2001; Huey & Berrigan, 2001; Deutsch *et al.*, 2008; Lelièvre *et al.*, 2011; Amarasekare & Savage, 2012). For example in ectotherms several important processes are maximized within a narrow range of body temperatures (termed the optimal temperatures), including locomotion (Stevenson, Peterson & Tsuji, 1985; Blouin-Demers & Weatherhead, 2008; Halliday & Blouin-Demers, 2015), growth rate (Angilletta, Steury & Sears, 2004), energy acquisition (Bergman, 1987), energy assimilation (Stevenson *et al.*, 1985; Angilletta, 2001) and reproductive output (Berger, Walters & Gotthard, 2008; Halliday & Blouin-Demers, 2014; Halliday & Blouin-Demers, 2015; Halliday, Thomas & Blouin-Demers, 2015). Moreover, temperature can modulate the density dependence of fitness, where negative density dependence is strongest at the optimal temperature and weakens as temperature deviates from the optimal temperature (Halliday & Blouin-Demers, 2014; Halliday *et al.*, 2015). Since the negative density dependence of fitness is a crucial aspect of density-dependent habitat selection (Fretwell & Lucas, 1969; Rosenzweig, 1981; Morris, 1988; Morris, 2011), changes in temperature should modify the density dependence of habitat selection in ectotherms.

In this study, we first examine how density-dependent habitat selection theory is affected by temperature to obtain temperature-dependent predictions. We then test these predictions using a controlled habitat selection experiment with red flour beetles (*Tribolium castaneum*). We use our previous results on the strength of fitness-density functions (Halliday & Blouin-Demers, 2014; Halliday *et al.*, 2015) to make specific predictions about how red flour beetles should select between habitats differing in the quantity of food as temperature changes. For the first time, this allows us to examine specifically how temperature affects the strength of density-dependent habitat selection in an ectotherm. Although temperature also impacts fitness and habitat selection in endotherms (e.g. Schwab & Pitt, 1991; Humphries, Thomas & Speakman, 2002), the impact tends to be much less pronounced and is thus restricted to more extreme variation in temperature. Ectotherms are affected even by small changes in temperature. The theory we develop is therefore applicable to ectotherms across all temperature variation, but only applicable to endotherms across very high-temperature variation.

Theory

From Morris (1988), fitness (W) in a habitat (i) can be modelled as:

$$W_i = \gamma \left[R_i - N_i p \frac{E_b}{E_i} \right] \qquad (1)$$

where γ is a scaling constant, R_i is the amount of resources in a habitat corrected by its renewal rate (Morris, 1988), which is equivalent to the fitness of an individual in the absence of competition. N_i is the population density in a habitat, E_b/E_i is the efficiency of extracting resources, consumption and conversion into descendants in habitat i relative

to the best habitat b, and p is the per capita demand on resources. Equation 1 can be simplified to a density-dependent fitness line (Morris, 1988), known as the fitness-density function, where per capita fitness decreases as density increases:

$$W_i = R_i - N_i U_i \qquad (2)$$

In this simplified equation, U is a combination of the per capita demand on resources and the extraction efficiency in habitat i. For ectotherms, we can add temperature dependence of resource acquisition and resource assimilation (T) to equation 2:

$$W_i = R_{Ti} - N_i U_{Ti} \qquad (3)$$

where R_{Ti} is the thermal reaction norm for fitness in a given habitat (i) in the absence of competition, and U_{Ti} quantifies temperature dependence of per capita demand on resources and extraction efficiency. Given that R_i (equation 2) is essentially the ability of an individual to convert the resources in habitat i into offspring in the absence of competition, it is a logical extension to use the thermal reaction norm for fitness in the absence of competition (R_{Ti}) to describe the temperature dependence of R_i.

U_{Ti} includes the temperature-dependent effects of both p and E_b/E_i. p should follow the same pattern as the thermal reaction norm for fitness because per capita resource demand (i.e. the amount of resources required per individual) will decrease as temperature deviates from T_o: food consumption decreases as temperature deviates from T_o (Stevenson *et al.*, 1985; Angilletta, Hill & Robson, 2002; Halliday & Blouin-Demers, 2016b). p will be highest at T_o and will approach zero as temperature deviates from T_o. E_i should similarly approach zero as temperature deviates from T_o due to the temperature dependence of resource extraction (Halliday & Blouin-Demers, 2016b).

If the thermal reaction norm for fitness is unknown, then proximate measures of fitness, such as fecundity, can be used to estimate the impact of temperature on fitness due to the coadaptation of thermal reaction norms for traits related to fitness (Halliday & Blouin-Demers, 2015). We assume that neither R_{Ti} nor U_{Ti} are linear; they should follow the general shape of the thermal reaction norm for fitness in the species: a gradual increase as temperature approaches T_o followed by a sharp decrease as temperature increases above T_o (Angilletta, 2006; Bulté & Blouin-Demers, 2006; Halliday & Blouin-Demers, 2015). Thus, as temperature deviates from T_o, fitness decreases and negative density dependence of fitness weakens (Halliday *et al.*, 2015).

Under an ideal free distribution, individuals are distributed between habitats to equalize fitness (Morris, 1988):

$$W_a = W_b \qquad (4)$$

where W_a is fitness in habitat a, and W_b is fitness in habitat b. We can then substitute the right side of the fitness-density function (equation 3) for W in each habitat

$$R_{Ta} - N_a U_{Ta} = R_{Tb} - N_b U_{Tb} \qquad (5)$$

and solve for N_a as previously done by Morris (1990):

$$N_a = \frac{R_{Tb} - R_{Ta}}{U_{Ta}} + \frac{U_{Tb}}{U_{Ta}} N_b \qquad (6)$$

Equation 6 can then be simplified to give the habitat isodar (Morris, 1988):

$$N_a = C + bN_b \qquad (7)$$

where C, the isodar intercept, is the difference in temperature-dependent highest potential fitness between the two habitats divided by temperature-dependent extraction efficiency and per capita resource demand in habitat a

$$C = \frac{R_{Tb} - R_{Ta}}{U_{Ta}} \qquad (8)$$

and b, the isodar slope, is the ratio of temperature-dependent extraction efficiency and per capita resource demand between the two habitats

$$b = \frac{U_{Tb}}{U_{Tb}} \qquad (9)$$

If temperature deviates from T_o in both habitats simultaneously, the isodar intercept will approach zero and the slope will approach one until habitat selection is equalized (individuals show equal preference for both habitats) or until habitat selection is density-independent.

To illustrate this theory, we used data from Halliday *et al.* (2015) (Fig. 1a). Briefly, fitness was the per capita number of eggs laid over 4 days by red flour beetles at five density treatments (10, 20, 30, 40 and 50 beetles), two food treatments (0.625 and 2.5 mL of wheat flour) and three temperature treatments (20, 25 and 30°C). In red flour beetles, $T_o = 30°C$ according to multiple measures of fitness (Halliday & Blouin-Demers, 2014; Halliday & Blouin-Demers, 2015; Halliday *et al.*, 2015). We opted to use egg laying rate as our metric of fitness because it is easy to measure and is correlated with the number of adult offspring produced, which is a more ultimate measure of fitness (rho = 0.42 and 0.49 at 25 and 30°C, respectively, between the number of eggs laid and the number of adult offspring produced from data in Halliday *et al.*, 2015).

The fitness data clearly demonstrate that the fitness-density function is strongest at T_o and weakens as temperature deviates from T_o. We built predictive isodars with these fitness-density functions (Fig. 1b), where each isodar is based on the assumption that beetles select habitats to maximize individual fitness (i.e. according to the IFD). We predicted that the slope of the isodar should be the steepest at T_o (strongest preference for high food) and should approach equality (slope of one) as temperature deviates from T_o. The intercept should similarly be highest at T_o and should approach zero as temperature deviates from T_o. Habitat selection at 20°C should be density-
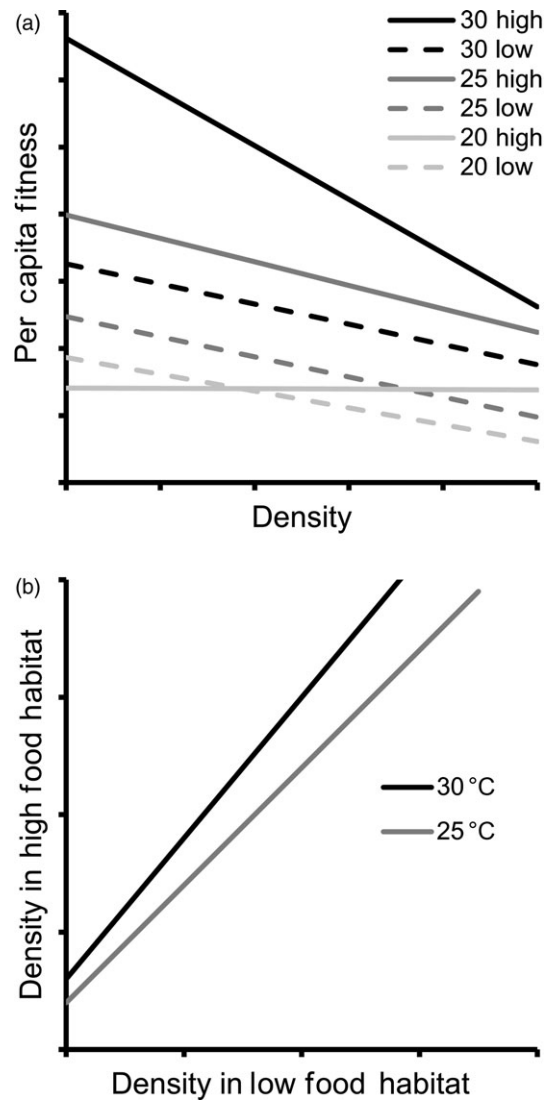

Figure 1 (a) Mean fitness-density functions, based on per capita eggs laid, under high and low food treatments at three temperatures (20, 25 and 30°C) for red flour beetles (*Tribolium castaneum*) (adapted from Halliday *et al.*, 2015). (b) Theoretical isodars predicting habitat selection based on temperature.

independent (i.e. no isodar) because the relationship between fitness and density was not statistically significant at 20°C (Halliday *et al.*, 2015).

We used red flour beetles to conduct controlled habitat selection experiments across a range of population densities and three temperatures to test these predictions. We also confirmed that fitness decreases as density increases and that the fitness difference between habitats weakens as temperature deviates from T_o by measuring egg production in each habitat while we documented habitat selection; we built updated predictive isodars at each temperature with these new data.

Materials and methods

Study species

We obtained a colony of 200 red flour beetles (*Tribolium casta-neum*) from Carolina Biological Supply Company (Burlington, North Carolina, USA) and kept beetles with ad libitum flour (95% all purpose flour and 5% yeast) at 30°C and 70% relative humidity under a 12:12 hour light:dark cycle. The beetles lived under these conditions for 18 months prior to our experiments and colony size increased to over 5000 individuals. Red flour beetles are small (length = 3-5 mm, mass = 1-4 mg), part of the Tenebrionidae family (darkling beetles) and widespread agricultural pests (Good 1936). For experiments requiring females only, we sexed beetles at the pupal stage (Good 1936) and kept female pupa separate from male pupa.

Habitat selection experiments

We set up experimental habitats in clear plastic containers (31 × 17 × 10 cm) with 1 cm of sand as a substrate. We created two food patches in each container (one at each end, with 20 cm between them) by attaching two glass slides together with tape (combined dimensions: 75 × 50 mm): a low food patch (0.625 mL of flour) and a high food patch (2.5 mL of flour). We used the same habitats in our previous habitat selection experiments (Halliday & Blouin-Demers, 2014; Halliday & Blouin-Demers, 2016a); the ratio of flour between habitats in the current experiment was selected based on the results from multiple food ratio treatments in a previous study (Halliday & Blouin-Demers, 2016a). We placed each container in an environmental chamber for 24 hours with a 12:12 hour light:dark cycle at a constant temperature. We placed beetles in the middle of each container and after 24 hours we counted the number of beetles on each side of the container. We then sifted the flour through a 250 μm sieve and counted the number of eggs that had been laid in each food patch. We used 10 replicates of five density treatments (10, 20, 30, 40 and 50 beetles) and three temperature treatments (20, 25 and 30°C), with either mixed groups (~1:1 sex ratio) or female-only groups in a fully factorial design (*n* = 300 containers). We used both mixed groups and female-only groups because male flour beetles emit aggregation pheromones (Suzuki, 1980) that can affect the ability of beetles to achieve an ideal-free distribution (Halliday & Blouin-Demers, 2016a). We therefore used female-only groups to obtain habitat selection patterns that only reflect the factors we manipulated, and we used mixed groups of beetles to obtain fitness data (number of eggs laid) and habitat selection data that are affected by aggregation pheromones in addition to the variables we manipulated. We randomly selected all beetles for each treatment, each beetle was only used once, and we assumed a 1:1 sex ratio in the mixed group of beetles based on the observed 1:1 sex ratio of pupa in our cultures (Halliday and Blouin-Demers *unpublished data*). We had a single environmental chamber, so we tested one temperature treatment at a time (from coldest to hottest). Although this experimental design added the potential confounding effect of time, there were logistical constraints owing to other concurrent experiments and we could not randomize temperature order to avoid this effect. All density treatments within a temperature treatment, however, were conducted simultaneously.

Statistical analyses

We analysed habitat selection data with isodars (Morris, 1988). We built isodars for each temperature treatment for both mixed groups and female-only groups with geometric mean regression in R (package: lmodel2; function: lmodel2; Legendre, 2014) with the number of beetles in the high food habitat as *Y* and the number of beetles in the low food habitat as *X*. We compared isodars using the confidence intervals around their intercepts and slopes, and with the fit of each model.

We calculated the per capita number of eggs laid (fecundity) in a habitat two ways: first, we calculated per capita fecundity by dividing the number of eggs in a habitat by the number of beetles in the treatment (overall per capita eggs); second, we calculated per capita fecundity by dividing the number of eggs in a habitat by the number of beetles counted in that habitat (habitat per capita eggs). We calculated per capita fecundity two ways because females laying eggs could be reacting to the number of beetles in the entire container or just to the number of beetles in a given habitat. We identified the factors governing overall per capita eggs using linear mixed effects models in R (package: nlme; function: lme; Pinheiro *et al.*, 2014) with density treatment, temperature treatment (as a factor), habitat type (low or high food) and all two- and three-way interactions as fixed effects, and with replicate as a random effect to account for habitats being paired within one treatment combination. We identified the factors governing habitat per capita eggs with a similar model, but with beetle density per habitat rather than the density treatment as a fixed effect. We compared models with bias-corrected Akaike's information criterion (package: qpcR; function: AICc; Spiess, 2014) and considered the best model the one with the lowest AICc value. These analyses allowed us to compare the intercepts and slopes for the fitness by density relationships for all six combinations of habitats and temperatures.

Finally, we built predicted isodars with the statistically significant fecundity by density relationships and compared these predicted isodars to the isodars we obtained in the habitat selection experiment.

Results

Female beetles demonstrated the strongest preference for the high food habitat at 30°C, a weakened preference at 25°C, and a statistically non-significant preference at 20°C (Fig. 2, Table 1). For female-only groups, the 95% confidence intervals around the intercepts of the isodars all overlapped with zero and the 95% confidence intervals around the slopes of the isodars were all greater than one. As temperature decreased, the slopes of the isodars approached one; the 30°C treatment had the steepest slope. Mixed groups of beetles never showed a statistically significant preference for either habitat (Fig. 2, Table 1). Models for female-only groups had higher R^2 values than mixed groups at all temperature treatments and R^2 generally decreased as temperature decreased.
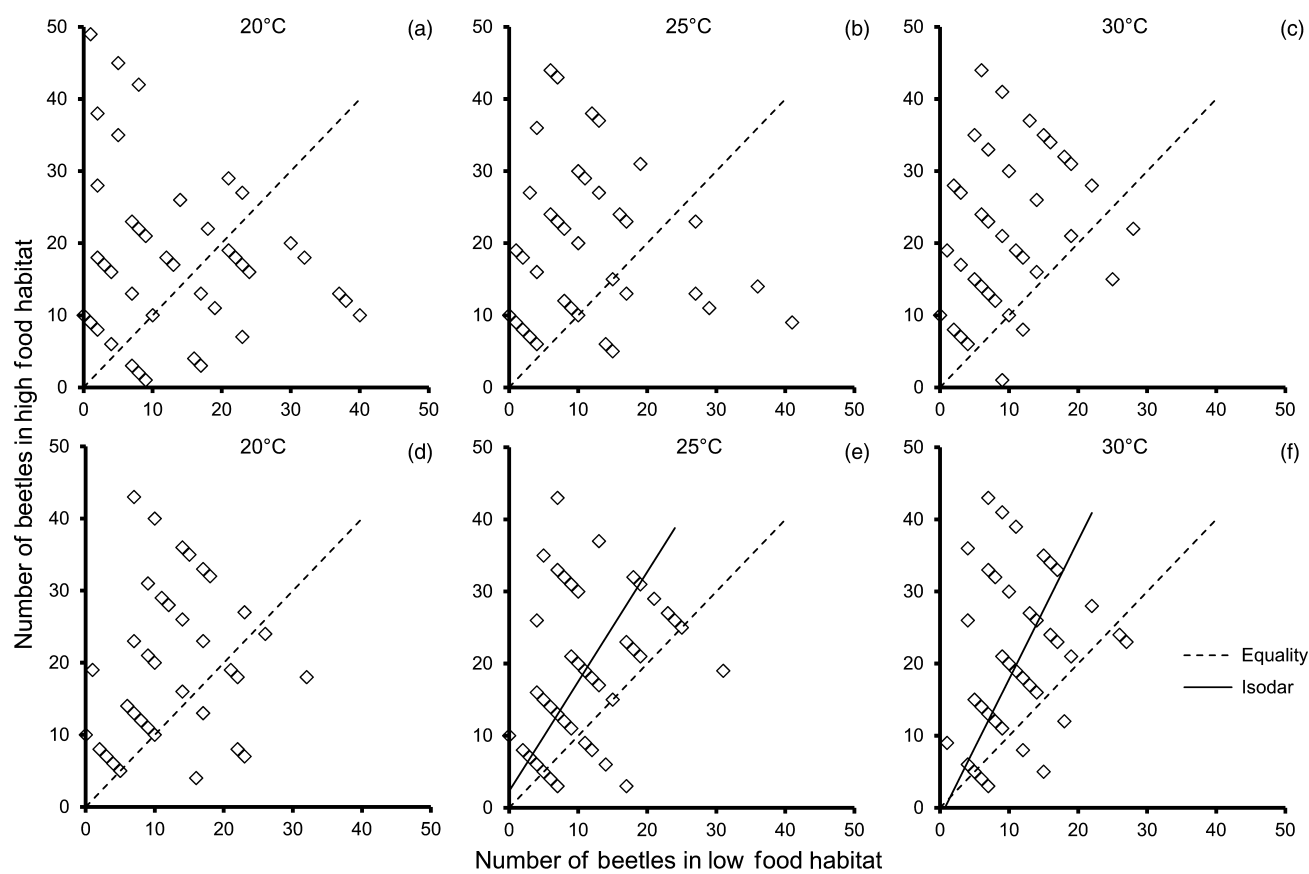
Figure 2 Experimental isodars for red flour beetles (*Tribolium castaneum*) selecting between habitats with high food and low food when temperature was 20°C (a, d), 25°C (b, e) and 30°C (c, f). The dashed lines represent equal selection of each habitat, and the solid lines represent statistically significant isodars. Isodars were built with mixed groups of males and females (a-c) and with female-only groups (d-f).

Table 1 Isodar equations, built via geometric mean regression, for habitat selection by red flour beetles (*Tribolium castaneum*) selecting between low and high food habitats across a range of densities at three temperatures. Y is the number of beetles in the high food habitat and X is the number of beetles in the low food habitat. Mixed Group refers to an equal sex ratio, and Female Groups refer to experiments with females only. CI represents the 95% confidence interval

Treatment	Isodar equation	Intercept CI	Slope CI	R^2	P
Mixed groups					
20°C	$Y = 30.57 - 1.04\ X$	27.31 to 34.90	−1.38 to -0.79	0.01	0.43
25°C	$Y = 32.44 - 1.24\ X$	29.30 to 36.62	−1.65 to -0.93	0.01	0.99
30°C	$Y = 5.78 + 1.70\ X$	0.92 to 9.46	1.29 to 2.46	0.07	0.06
Female groups					
20°C	$Y = 0.78 + 1.43\ X$	−4.72 to 4.95	1.09 to 1.89	0.06	0.08
25°C	$Y = 2.31 + 1.52\ X$	−2.92 to 6.29	1.16 to 2.00	0.10	0.03
30°C	$Y = -1.34 + 1.92\ X$	−7.69 to 3.51	1.47 to 2.52	0.12	0.01

Overall per capita fecundity had the strongest negative density dependence at 30°C (Fig. 3; Supplementary material Table S1) and was stronger in high food patches than in low food patches (high food patch: per capita fecundity = 1.14 − 0.02 × density; low food patch: per capita fecundity = 0.50 − 0.01 × density). At 25°C, the negative density dependence of overall per capita fecundity was weaker in the high food patches and was absent in the low food patches (high food patch: per capita fecundity = 0.44 − 0.01 × density; low food patch: not statistically significant). Negative density dependence of overall per capita fecundity disappeared at 20°C in both habitats.

The negative density dependence of habitat per capita fecundity was only statistically significant at 30°C (Fig. 3; Supplementary material Table S2). At 30°C, habitat per capita fecundity was higher in the high food patch, but negative
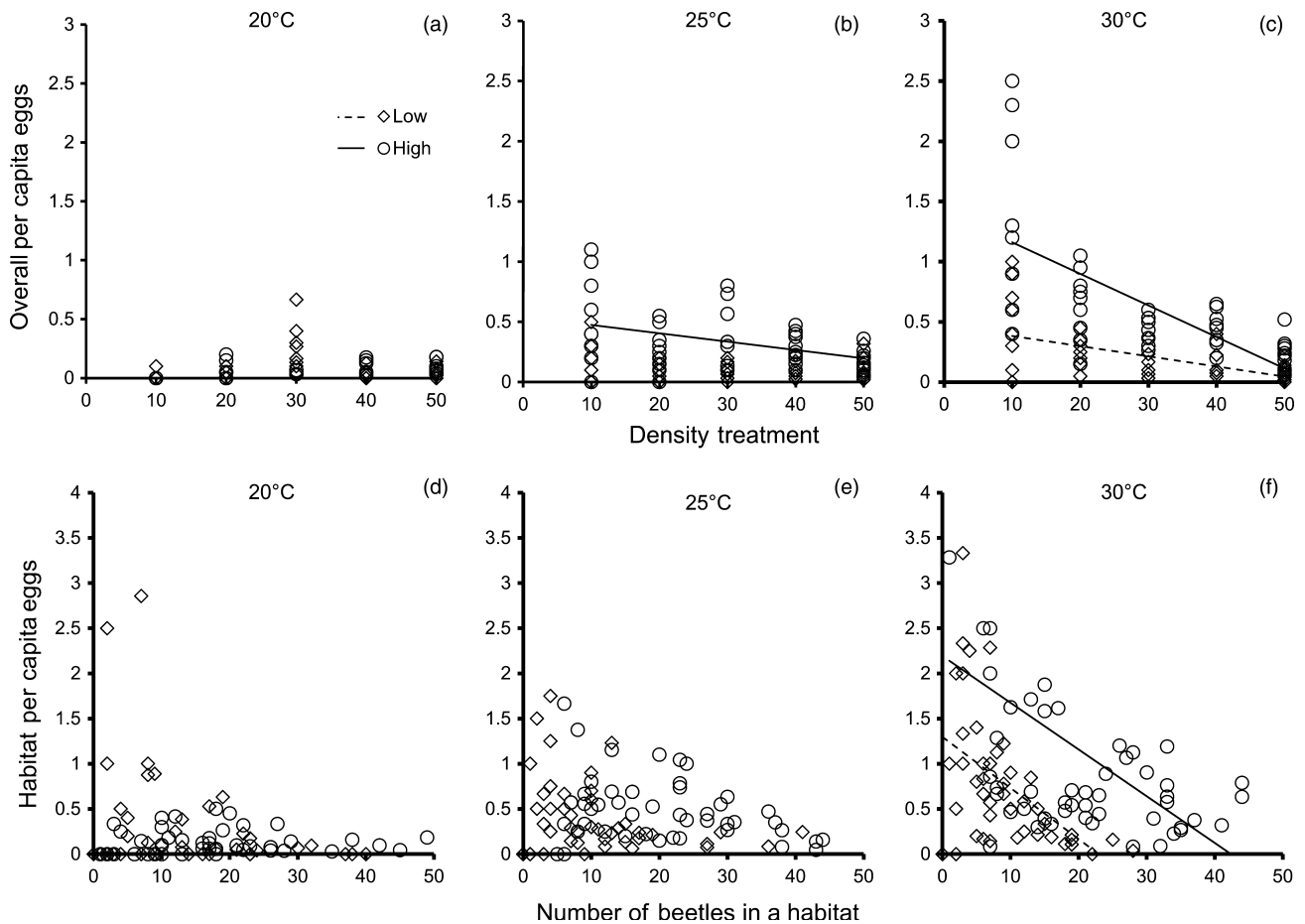
Figure 3 Overall per capita number of eggs (a-c) and habitat per capita number of eggs (d-f) laid by red flour beetles (*Tribolium castaneum*) in low food and high food habitats over 24 hours at different density treatments when temperature was 20°C (a, d), 25°C (b, e) and 30°C (c, f). Overall per capita eggs were calculated as the number of eggs in a habitat divided by the total number of beetles in the treatment. Habitat per capita eggs was calculated as the number of eggs in a habitat divided by the number of beetles in that same habitat. The dashed lines represent a statistically significant effect of density on fecundity in low food habitats, and the solid lines represent the same in high food habitats.

density dependence was equal between habitats (high food patch: per capita fecundity = 2.44 − 0.07 × density; low food patch: per capita fecundity = 1.12 − 0.07 × density).

We could only build predicted isodars based on the fecundity data for the 30°C treatment because the 20 and 25°C treatments had non-significant fecundity by density functions. Habitat per capita fecundity predicted an isodar with slope equal to one and an intercept >1, and overall per capita fecundity predicted an isodar with slope >1 and an intercept >1 (Fig. 4). The measured isodar for 30°C (Figs 2 and 4), with slope >1 and intercept = zero, overlapped with these predicted isodars at densities between 5 and 15 beetles in the high food habitat, but had fewer beetles than predicted in the high food habitat at low densities and more beetles than predicted in the high food habitat at high densities. The 95% CI around the isodar overlapped with the predicted isodar based on overall per capita fecundity (Fig. 4), and overlapped with the predicted isodar for habitat per capita fecundity at all densities but the lowest (Fig. 4).

Discussion

In agreement with our predictions, density-dependent habitat selection weakened as temperature deviated from T_o, for both the slope of the relationship and for the fit of the model. This indicates that the weakening of density dependence as temperature deviates from T_o does indeed appear to affect habitat selection by beetles. Habitat selection at 20°C was density-independent, in agreement with the prediction of Morris (1988) that density-independent fitness functions should lead to density-independent habitat selection. It is unsurprising that density dependence disappears at 20°C in red flour beetles because, although they lay eggs, none of these eggs successfully develop into adults (Howe, 1956; Halliday *et al.*, 2015). Therefore, the non-significant fitness-density function at 20°C is indicative of zero fitness at that temperature. Beetles at 25°C had fitness that was intermediate to that at 20°C and 30°C, and the strength of density dependence was similarly intermediate to that at 20°C and 30°C. Accordingly and as predicted,
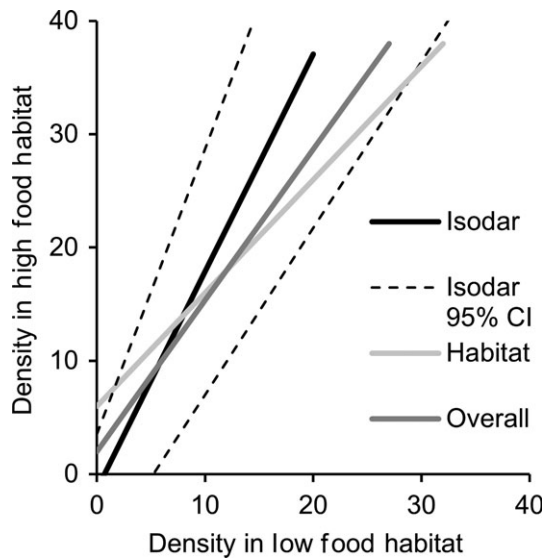
Figure 4 Predicted isodars for habitat and overall per capita eggs compared with the measured isodar for red flour beetles (*Tribolium castaneum*) selecting between high and low food habitat at 30°C. The dashed black lines represent the 95% CI around the isodar.

this intermediate fitness-density function led to an isodar that was again intermediate to the isodars at 20°C and 30°C.

Despite the support for the effect of temperature on the strength of isodars, predicted isodars built with fecundity data (Figs 1 and 4) did not accurately reflect patterns in habitat selection (Fig. 4). According to the fecundity data, isodars at both 25 and 30°C should have had intercepts greater than zero, whereas all isodars that we measured had intercepts not different from zero. The slopes that we measured were also steeper than predicted based on fecundity data (Fig. 4). Although fecundity is correlated with the number of adult offspring produced, this correlation seems too weak to predict the habitat selection of flour beetles accurately. Future studies should use absolute fitness measures, such as the number of adult offspring produced or lifetime reproductive success, to create predictions for density-dependent habitat selection and to then test these predictions with habitat selection data.

The number of eggs laid is only one possible proximate measure of fitness and different fitness proxies could have different thermal reaction norms. Since T in equation 3 is explicitly linked to the shape of the thermal reaction norm for fitness, predictions may vary slightly depending on which metric is used to estimate fitness. Although according to the thermal coadaptation hypothesis (Bennett, 1980; Blouin-Demers, Weatherhead & McCracken, 2003; Halliday & Blouin-Demers, 2015) all thermal reaction norms closely related to fitness should have similar shapes with similar optima, differences in the rate of change from critical thermal minimum to the optimal temperature could lead to different predictions when comparing habitats that differ in just a few degrees, such as our 25 and 30°C treatments. Future work could examine other fitness-density functions and their ability at predicting habitat selection. For example rates of energy acquisition and assimilation

vary with temperature (Angilletta, 2001), and may also decrease as density increases due to increased competition for food. These fitness metrics may therefore also be good predictors of habitat selection given the relationship between habitat suitability and energy acquisition (Morris, 2014).

While temperature has a well-established impact on fitness and habitat suitability, it is also possible that temperature may impact the underlying assumptions of ideal free habitat selection. For example decreased temperature causes movement to be slower and therefore increases travel time between habitats. While this slower movement may not have any energetic cost, time may be considered a cost if there are other activities that individuals could be doing instead of travelling and if these activities offer greater fitness rewards. In the context of our experiments, we do not think that decreased velocity would lead to a violation of assumptions of the IFD because the main cost in our system should be energy rather than time. The other assumptions of the IFD are ideal knowledge and equal competitive abilities. Ideal knowledge should not be violated at lower temperatures, but it will take individuals longer to sample habitats. Competitive abilities should be affected by temperature because the ability of ectotherms to extract resources decreases as temperature deviates from the optimal temperature; snakes, for instance, fight over food more intensely at the optimal temperature than at sub-optimal temperatures (Halliday & Blouin-Demers, 2016b). Changing temperature, however, should not affect the equality of competitors because temperature should affect all individuals equally. Although temperature can affect the sexes differently (e.g. Ørsted, Schou & Kristensen, 2017), we were unable to isolate the effect of sex in this study due to the strong influence of male aggregation pheromones on habitat selection (Halliday & Blouin-Demers, 2016a). We therefore believe that the assumptions of the IFD were not violated by our temperature treatments.

The presence of males weakened patterns of density-dependent habitat selection, as we expected based on our previous work (Halliday & Blouin-Demers, 2016a). This is likely due to the aggregation pheromones that are released by males (Suzuki, 1980). In the presence of males emitting these pheromones, all individuals may select habitat based on the location of pheromones rather than the factors that we manipulated (Halliday & Blouin-Demers, 2016a). Despite this extra noise in the data, beetles matched our general predictions (i.e. weakening relationship as temperature decreases), but the trends were not statistically significant and were much stronger when we used females only. Using mixed groups of beetles allowed us to obtain fitness data while simultaneously measuring habitat selection, something we had not attempted previously. A potential issue with our mixed groups of beetles is that we assumed that we had equal numbers of males and females, but had no way of verifying the actual sex ratio. This uncertainty in sex ratios likely caused increased variability in our fecundity data, especially in the lower density treatments where there was an increased probability of a biased sex ratio due to the small number of individuals in a treatment.

Our isodars explained relatively small proportions of variation ($R^2 \leq 0.12$) in our habitat selection data. Much of the spatial distribution of beetles was likely explained by factors other

than our low and high food habitats. In our previous study where each habitat was at a different temperature, isodars explained much higher variance ($R^2 = 0.44$; Halliday & Blouin-Demers, 2014), thereby demonstrating that our experimental approach is appropriate for manipulating factors affecting the spatial distribution of this species. Given that food is just one aspect of habitat suitability and that other factors such as temperature may be more important in the habitat selection of this species, the R^2 values that we recorded here likely reflect the importance of these different factors in dictating habitat suitability.

This study builds on our previous work (Halliday & Blouin-Demers, 2014), but it is distinct in important ways. In our previous study, we measured habitat selection of beetles between two habitats that varied in both temperature and food abundance simultaneously. In this study, we allowed beetles to select between two habitats that differed in food abundance, and we repeated this manipulation at three temperatures to determine if patterns of habitat selection weaken as temperature deviates from the optimal temperature. While these differences may appear small, the implications for theory are important. It would have been impossible to test the predictions that we developed in this study with the data from our previous study because food abundance and temperature were manipulated simultaneously. In addition, because we separated the effect of food abundance from the effect of temperature, the predictive power of this study is much higher than the predictive power of our previous study.

Our results have striking implications for our understanding of habitat selection by ectotherms. Since density dependence is strongly affected by temperature in ectotherms, field studies examining habitat selection could have confounding effects of temperature that could mask or negate the effect of density. It becomes increasingly difficult to detect density dependence as temperature departs from T_o. For example a study conducted when environmental temperatures are well below T_o, such as the 20°C treatment in our study, could lead to the conclusion that a species does not select habitats in a density-dependent fashion, or that the species has equal preference for two habitats that actually have very different food availabilities. Moreover, in temperate ecosystems with short summers, density dependence may only be important during the few warm weeks when environmental temperatures approach T_o. It is therefore imperative to consider explicitly the role that temperature plays on density dependence when working with ectotherms. We worked at temperatures below T_o, but we assume a similar pattern would emerge above T_o given the shape of thermal reaction norms, but future work should confirm this.

Acknowledgements

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Supporting Information

Additional Supporting Information may be found in the online version of this article:

Table S1. Model selection and final model output for linear mixed effects models examining overall per capita eggs laid (total eggs/population density) by red flour beetles (*Tribolium castaneum*) in high and low food habitats at different population densities and at three temperatures.

Table S2. Model selection and final model output for linear mixed effects models examining per capita eggs laid in a habitat (eggs in a habitat/beetles in a habitat) by red flour beetles (*Tribolium castaneum*) in high and low food habitats at different population densities and at three temperatures.