

Greener on the other side of the fence: density-dependent habitat selection by a unicellular alga

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ABSTRACT

Background: When populations have the opportunity to occupy multiple habitats, individuals that move to habitats with higher mean fitness will expect to produce more descendants than will individuals that are incapable of such adaptive movements. The ability to make an optimal decision depends on habitat quality, the quality and reliability of the cue for habitat choice, and the ability of the organism to respond to the cue appropriately.

Questions: Do simple motile organisms select habitats that maximize fitness? Do they move such that densities in different habitats equalize fitness? Does the ability to select between habitats depend on the difference in habitat quality?

Organism: *Chlamydomonas reinhardtii*, a motile, chemo- and phototactic single-celled alga.

Experiments: We introduced clonal populations, at 15 different densities, into either shaded or fully lighted aqueous habitats in one side of Petri dishes. The other side of the dishes contained either unused (high quality) or used (lower quality) growth media. We estimated fitness in control dishes containing a single habitat. We used the estimates to predict the density expected when algae could choose between light and shaded habitats in the same dish.

Results: Fitness declined linearly with increasing density. Mean fitness was higher in control light than in control shade. Patterns of density and fitness in treatment dishes depended on which habitat the cells were introduced into, and on the quality of growth media. Cells were more abundant in light than in shade when introduced into the light side of habitat-selection dishes containing unused media. There was no difference in fitness between habitats in this treatment. Cells introduced into the shaded side with unused media attained similar densities in both habitats. Fitness was higher in light. Cell densities in the two habitats were also similar in both treatments containing used media. Fitness was higher in the light habitat in each of these treatments.

Conclusion: Motile non-sentient species with simple sensory abilities can move among habitats to increase fitness. They can also attain distributions that equalize fitness differences between habitats. The threshold for adaptive habitat choice depends on the cues for habitat quality and the costs of habitat selection. Whether fitness and density differ between habitats depends on the quality of the environment in which habitat selection occurs.

Keywords: algae, *Chlamydomonas reinhardtii*, density, dispersal, fitness, habitat choice, ideal free distribution, phototaxis, signal detection.

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INTRODUCTION

Virtually all organisms occupy heterogeneous environments where habitat quality varies through space and time. Most, if not all, of these organisms possess adaptations such as sensory capability and mobility that enable non-random use of habitats varying in quality. Non-random use is frequently modelled as an adaptive strategy maximizing fitness (e.g. Fretwell and Lucas, 1969; Rosenzweig, 1981; Morris, 1988). The ability of any given strategy to maximize benefits over costs of habitat selection depends on such factors as density, degree and timing of mobility, costs of habitat assessment and dispersal, sensory cues, decision rules, and the amount and value of information that individuals possess on their various choices. Habitat selection also depends on cognitive ability, development and maternal effects (e.g. Grafen, 1988; Stamps, 2006), exposure to natal habitat (Davis and Stamps, 2004), life history, sensory skills, and condition (del Mar Delgado *et al.*, 2010; Remy *et al.*, 2011). Habitat choice varies with social organization and public information (Doliguez *et al.*, 2002; Danchin *et al.*, 2004; Cote and Clobert, 2007), interspecific interactions (Morris, 1988, 1999; Morris *et al.*, 2000), distance, landscape context (Resetarits and Binckley, 2013), and the information content of signals on habitat quality (Clobert *et al.*, 2009).

With so many co-varying factors, we can expect a continuum of outcomes. At one extreme, organisms achieve perfect habitat selection that equalizes fitness among habitats (e.g. Fretwell and Lucas, 1969; Fretwell, 1972; Rosenzweig, 1981; Morris, 1988). At the other extreme, individuals move randomly from one habitat to another. Regardless, any motile organism with sensory capacity should fit somewhere within the continuum of possible strategies of habitat selection. The location along the continuum should vary with differences in habitat quality and with the costs of selecting one habitat over others. Thus, we wished to determine whether *Chlamydomonas reinhardtii*, a single-celled motile alga, would exhibit different strategies of habitat selection as we experimentally manipulated population density, light regime, and nutrient concentrations. The aims of this study were as follows:

- to demonstrate, by theory and controlled experiments, how to investigate multiple factors influencing habitat selection;
- to determine whether changing the difference in quality between habitats alters the precision with which organisms select habitat;
- to ascertain whether relatively simple motile organisms use density-dependent habitat selection to maximize fitness.

We begin with a brief review of the expected relationships between signal-detection and habitat-selection responses. We then review *C. reinhardtii*'s natural history and physiology as it impinges on signal detection and habitat choice. We describe experiments designed to test for habitat selection under varying expectations of fitness. The experimental design allows us to develop emergent hypotheses that predict the density-dependent habitat selection by *C. reinhardtii*. We test each prediction and re-assess the causes of variation in habitat selection. We conclude by discussing lessons that an improved understanding of habitat selection can teach us about population dynamics and spatial distribution.

Incomplete information and signal detection

Organisms obtain information from the environment through a variety of sensory abilities. The organism's ability to respond appropriately depends on the quality and variation in

information, how it is processed and integrated into action, and the value it affords to the individual. In the case of habitat selection, we can imagine an individual making a decision to either exhibit no habitat choice (e.g. random diffusion) or to differentially occupy habitats differing in quality. The individual's challenge is to develop a decision rule corresponding to a habitat-selection strategy that maximizes fitness.

We illustrate the problem of developing a decision rule in Fig. 1, where we assume that habitat selection can occur between high-fitness (H2) and low-fitness (H1) habitats of equal size. The two habitats are oriented along a sensory gradient that the organism can detect and which it uses as a cue to discriminate between random use and habitat selection. Mean fitness is greater in habitat 2 than it is in habitat 1. The habitat-selection strategy emerges from a decision rule that sets a threshold value of the gradient for habitat occupation. Individuals that search the gradient and find a cue greater than a threshold value (α) occupy the habitat in which the high value of the cue was encountered. Individuals that never encounter a cue as large as α do not prefer one habitat over the other and occupy each relative to its availability [i.e. fine-grained habitat use (Levins, 1962)]. Differences in the quality of the two habitats, and their relationships with the sensory gradient, determine how effectively any given threshold cue will differentiate between them.

The optimal α will correspond to the minimum value of the cue where habitat selection exceeds the fitness expectation achieved by being non-selective. Given our assumptions, habitat selection occurs whenever

$$P_{CA}W_2 + P_{FA}W_1 > (P_{CR} + P_M) \left(\frac{W_1 + W_2}{2} \right) \quad (1)$$

where P -values from left to right refer to the probabilities of correctly accepting H2, falsely accepting H1, correctly rejecting H1, and missing H2, respectively, and W_i is the fitness expectation in habitat i (derivation in evolutionary-ecology.com/data/2869Appendix.pdf; *Ideal free habitat selection when individuals err in their habitat choices*). The left-hand side of inequality (1) is the fitness accrued by selectively occupying H2 and H1 above α and the right-hand side is the fitness obtained through non-selective occupation of both habitats (the product of the probabilities of rejection and misses multiplied by mean fitness). Please note that inequality (1) refers to the expected fitness of an individual accepting versus rejecting habitat selection based on the value of the cue. The inequality does not represent the proportion of individuals in the population occupying one habitat or the other.

Discrimination for a given α is more likely when mean habitat quality and differences in quality between habitats are high (Fig. 1, top panel) than when mean habitat quality and differences in quality between them are low (Fig. 1, bottom panel). Regardless, individuals will frequently occupy both the habitat with high quality (correct acceptance) and that of lower quality (false acceptance). Thus, an ecologist's ability to detect habitat selection is complicated when habitats emitting similar cues make discrimination difficult. Problems of discrimination are exacerbated when the fitness difference between habitats is small. Small differences in fitness between habitats reduce the benefit achieved by an individual through correct decisions and the cost it accrues through errors. The ability to achieve the optimal solution also depends on whether individuals encounter and properly interpret the cue for habitat quality (e.g. Robertson *et al.*, 2013). If many individuals fail to detect or interpret the cue, then their indiscriminate use of habitat could swamp any differences in density associated with the optimal habitat-selection decisions of others. An ecologist observing such a population might mistakenly conclude that none of the individuals is capable of habitat

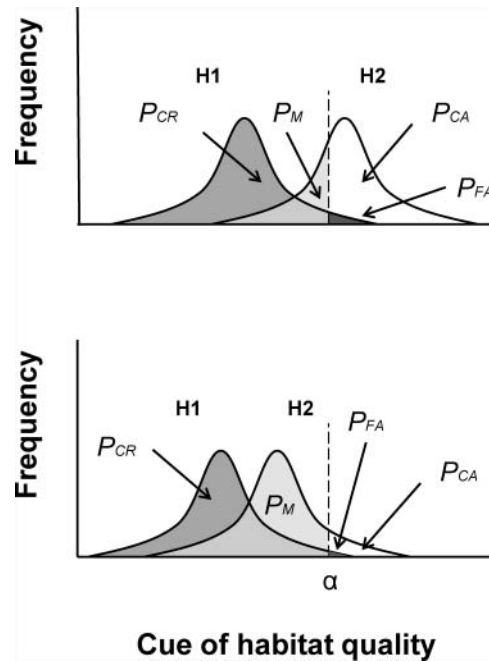


Fig. 1. An illustration of the ‘discrimination problem’ that organisms face when setting a threshold cue (α) for habitat selection. Habitat 2 (H2) is of higher mean quality than habitat 1 (H1). When both habitats are of reasonable quality and different from one another (top), the probability of missing an opportunity to occupy the better habitat ($P_{CA} - P_M$) for a given level of discrimination is less than when mean quality and differences between habitats are reduced (bottom). Note that, in both instances, some individuals will falsely occupy the poorer habitat. Shading indicates probabilities of correctly and falsely discriminating between the two habitats; P_{CA} = probability of correctly occupying H2, P_M = probability of missing occupation of H2, P_{CR} = probability of correctly rejecting occupation of H1, P_{FA} = probability of falsely occupying H1 (adapted from Stephens, 2007; see also Robertson *et al.*, 2013).

choice. The problem of detecting habitat selection is likely to be further compromised when habitat quality deteriorates with density. Such difficulties may explain variation in habitat use by natural populations and suggest that field ecologists might profitably switch to controlled experiments when testing theories of habitat selection.

MATERIALS AND METHODS

Natural history of *Chlamydomonas reinhardtii* as it affects habitat selection

Chlamydomonas reinhardtii is a remarkable 10- μm green alga that exhibits a variety of phototactic and chemotactic capabilities associated with the differential beating of its two flagellae (e.g. Harris *et al.*, 2009). Cells can detect and reorient to a single photon, and swim quickly (100–200 $\mu\text{m} \cdot \text{s}^{-1}$) towards light (Harris *et al.*, 2009). In the dark, cells switch repeatedly between synchronous beating of their flagellae, which causes helical swimming along a linear axis, to short periods of asynchrony. One flagellum beats more rapidly than the

other during asynchrony and sharply reorients cell direction (Polin *et al.*, 2009). The resulting random walk causes populations to diffuse along concentration gradients at speeds of approximately $10 \mu\text{m} \cdot \text{s}^{-1}$ (Polin *et al.*, 2009). When exposed to light, phototaxis can be either positive or negative depending on light and culture conditions (Harris *et al.*, 2009). Cells exposed to high and low light levels appear to switch between positive and negative phototaxis in order to regulate photosynthesis that alternately reduces (high photosynthetic rate) and oxidizes (lower photosynthetic rate) cytoplasm and reduction-oxidation homeostasis (Harris *et al.*, 2009). Cells with oxidized cytoplasm exhibit positive phototaxis; those with reduced cytoplasm exhibit negative phototaxis (Wakabayashi *et al.*, 2011). Chemotaxis is similarly sophisticated and depends, among other factors, on whether cells are in a vegetative or gametic state (Govorunova and Sineshchekov, 2005). Signal transduction in response to phototactic and chemotactic stimuli is likely to occur along multiple pathways that are subject to both the internal state of the cells and extracellular concentrations of chemo-attractants. Thus, an experiment that exposes *C. reinhardtii* to pairs of habitats differing in light intensity and quality of the growth medium should vary fitness prospects associated with habitat selection. The experiment should also alter the value of information, and thereby elicit a variety of habitat-selection strategies.

Algal phototactic habitat selection is complicated by acclimation of their photosystems to different light intensities (Bonente *et al.*, 2012). Cells are stressed when light intensity varies because photo-acclimation involves a variety of changes in gene expression and subsequent protein synthesis and degradation (Bonente *et al.*, 2012). The adaptive value of emigration from one light regime to another must therefore include acclimation costs. Similarly, the adaptive value of remaining in a habitat, or moving to another, must include the loss in fitness associated with signal transduction and ‘directional’ beating of the flagellae.

Experimental design

We used narrow, impervious wax-based barriers to partition six 100-mm glass Petri dishes into two equal halves. We then introduced a randomly chosen density of 36-hour-old *C. reinhardtii* cultures living in 15 mL of aqueous growth medium into one of the two halves of each dish (Fig. 2; full details of all protocols are provided in the appendix, evolutionary-ecology.com/data/2869Appendix.pdf; *Detailed description of experimental protocols*). We placed 15 mL of freshly prepared medium on the other side. The side with algae thus contained ‘used’ media; the other side contained ‘unused’ media. We placed the dishes in a growth chamber with a 12 h light/dark cycle, removed the barriers, and covered one-half of each dish with two layers of black fibreglass micromesh. The mesh reduced light intensity from 2120 ± 160 (mean $\pm$ s.d.) to 240 ± 35 lux ($n = 144$). We created three light (no mesh) and three shade (entire dish covered by mesh) controls at the same time.

We inserted a new barrier at 12 h and measured the optical densities of the cells by spectrophotometer absorbance in each half of all dishes. We calibrated optical densities to cell densities with haemocytometer counts (evolutionary-ecology.com/data/2869Appendix.pdf; *Calibrating cell density*). We used these values to reveal the densities in each habitat achieved by habitat selection. Densities were re-measured at 24 h and again at 48 h (Fig. 2). We used the ratio of the 48 h to 24 h densities to estimate fitness (see ‘Fitness assays’ below). We repeated the protocol to achieve 15 different target densities, before replicating the entire experiment with used media on both sides of the barrier ($n = 120$ populations yielding 240 density estimates and 120 fitness estimates).

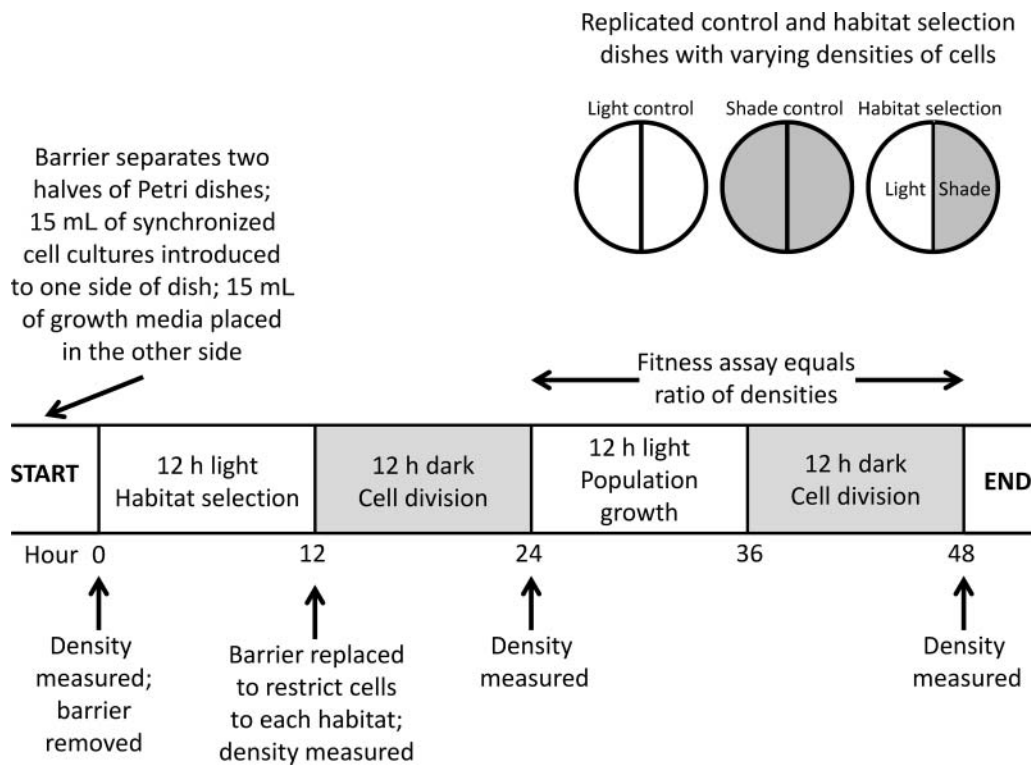


Fig. 2. The basic design and time course of experiments assessing the selection of 'light' versus 'shade' habitats by the single-celled alga, *Chlamydomonas reinhardtii*. Circles in the upper right represent caricatures of control and treatment Petri dishes used in the experiments.

Diffusion test

Our tests for adaptive habitat selection assume that: (1) 12 h is enough time for the cells to select habitat (see evolutionary-ecology.com/data/2869Appendix.pdf; *Evidence that habitat selection can occur in less than 12 h*); (2) the light versus shade treatments created rich and poor habitats respectively; and (3) algal distributions differed from those expected by diffusion of the media between the two halves of the Petri dishes. If the media failed to diffuse within 12 h, then (1) cells diffusing with the media might not encounter the alternative habitat, and (2) habitat selection for light versus shade in the first experiment might be confounded by differences in nutrient concentrations between the 36-hour-old cultures on one side, and the freshly prepared media on the other.

We tested for these possibilities by pipetting a 15-mL solution of media coloured with a single concentration of non-reactive dye into one-half of 12 different Petri dishes. We pipetted 15 mL of standard media into the other half of the dishes. We evaluated the time-dependent pattern of diffusion by measuring the optical absorbance of eight 220- μ L paired samples. Samples were collected from each side of the dishes at one-hour intervals for 12 h. We tested for differences between the two halves of the dish with paired *t*-tests ($n = 8$ for each of the 12 tests). We reasoned that the time when we were unable to detect a difference in absorbance would correspond with that required for diffusion to 'homogenize' the media.

Fitness assays

Cells synchronized on a 12 h light/dark cycle divide only during the dark (Harris *et al.*, 2009). So we estimated fitness as the per capita population growth rate achieved between 24 and 48 h (density at 48 h [N_{48}] divided by the density at 24 h [N_{24}]). Use of the 24–48 h time period guarantees that the growth estimates represent fitness achieved after the cells had completed any habitat choice. We rejected a fitness assay based on cell division between 12 h and 24 h because it would include fitness obtained by cells that occupied both habitats during the previous photosynthesis and habitat-selection phase of the experiment.

Theory and predictions

Let us first consider cells introduced in light. The cells' challenge is to assess cues of habitat quality, discriminate one habitat from the other, and 'choose' a strategy. One strategy is to remain in light and pay the cost of resisting diffusion. The alternative strategy is for cells to diffuse into shade where they accrue lower fitness and the cost of acclimating to lower light. We assume, for simplicity, that diffusing cells enter and acclimate to the shaded habitat once only. We further assume that cells resisting diffusion never cross into shade. We retain similar assumptions for all following models. Continuous diffusion and acclimation would inflate costs but should not alter the general predictions emerging from our model.

The distribution of cells between the two habitats at evolutionary equilibrium will correspond to those densities such that the fitness of cells in light equals the fitness of cells diffusing between habitats. Cells in light will thus include those that resist diffusion by staying in light with cost, and those that diffuse between the two habitats (one-half of these remain in light without cost). The fitness prospects (W_i) of each option are equal when

$$W_L - D - b_L(N_L + N) = \frac{[W_L - D - b_L(N_L + N)] + [W_S - A - b_S N]}{2}, \quad (2)$$

where subscripts denote light and shade habitats, b_i is the linear decline in fitness in habitat i , D is the density-independent loss in fitness associated with resisting diffusion, A is the density-independent loss in fitness caused by acclimating to shade habitat, N_L is the density of habitat selectors in the light habitat, and N is population density of diffusers (equal in both habitats). The expected total density in light is given by the system's isodar, the set of densities such that the fitness of each strategy is equal (Morris, 1987, 1988),

$$N_L + N = \frac{W_L - D - W_S + A}{b_L} + \frac{b_S}{b_L} N_S, \quad (3)$$

where, for clarity, we use a subscript to denote the number in shade ($N_S = N$).

Cells that interpret the cue of light habitat correctly should adopt the strategy of remaining in that habitat as long as they achieve higher fitness than do individuals diffusing into shade. The density of cells at equilibrium would be biased towards the light habitat and demonstrate the capacity for adaptive movement (Abrams, 2000; Cressman and Křivan, 2010) and habitat selection. Perfect assessment of habitat quality, perfect discrimination between habitats, and perfect resistance to diffusion when warranted by net fitness would yield an ideal distribution identified by the isodar.

It is possible that the cells might do even better. They might be able to distinguish light from shade habitat and occupy each one preferentially. The expected fitness at equilibrium would be the same in each. Intriguingly, the isodar for this situation is indistinguishable from that for diffusion because it yields the same intercept and slope,

$$N_L = \frac{W_L - D - W_S + A}{b_L} + \frac{b_S}{b_L} N_S. \quad (4)$$

If the cost of resistance to diffusion equals that of acclimation, these terms cancel in equation (4), and the isodar yields an ideal free distribution (IFD).

Let us now consider cells released in shade. These cells are immediately challenged with the cost of acclimation. Assuming that cells acclimate prior to diffusion towards light, cells diffusing into the lighted half of the dish will need to re-acclimate to its higher light intensity. These cells thus acclimate twice, once upon release in shade and again upon diffusion into light. We assume that the ‘reverse’ acclimation accrues the same cost as the original acclimation to shade. If the cells ‘choose’ to remain in the light, then they will need to pay the additional cost of resisting diffusion. The result is a lower initial density in the lighted habitat, i.e. with diffusion,

$$N_L = \frac{W_L - D - W_S - A}{b_L} + \frac{b_S}{b_L} N_S. \quad (5)$$

Finally, imagine that we repeat the two treatments of release in light or release in shade with lower-quality medium. Habitats of lower quality are shifted relative to fitness cues (Fig. 1). Three outcomes are likely. First, cells may retain the same ‘discrimination rule’ used with higher-quality medium. The likelihood of correctly remaining in light is reduced, as is the probability of differential habitat selection (compare the top and bottom panels in Fig. 1). Second, the probability of habitat selection may be reduced because discrimination between habitats has become more difficult. Discrimination may become compromised, for example, if nutrient or chemical changes in the media alter signal transduction. Third, the reduced quality of the media may influence habitat differences in fitness, and thus the intercepts and slopes of the isodars.

We thus predict that:

1. Differential habitat selection will be most favoured in treatments when cells acclimated to full light are introduced into the light habitat.
2. Habitat selection will be less likely to occur when cells are introduced into shade habitat, or when treatments are conducted with lower-quality growth media.
3. The ability to detect these effects will depend on acclimation and diffusion costs (and whether they are constants as modelled in equations 2–5), and any differences in the relationships between fitness and density associated with changes in habitat quality.

Tests for habitat selection

We estimated the relationships between per capita population growth rates and cell density by geometric mean regressions in R [lmodel2 package, 2.15.2 (R Development Core Team, 2008)]. We used those functions from the controls to predict the densities in each habitat expected

if habitat selection equalized fitness. The predicted densities are the habitat isodars. These predictions include acclimation to shade because all cells were initially cultured in light. The predictions do not include costs of resisting diffusion or those associated with re-acclimation to light (evolutionary-ecology.com/data/2869Appendix.pdf; *Fitness assays*). We then contrasted the actual relationships from the habitat-selection treatments with those predicted from the controls. We evaluated the fit of the models to data by verifying that the distributions of residuals were not different from expectation assuming a normal distribution. We concluded the experiments by using paired *t*-tests to assess whether fitness was different between the initial half of the Petri dishes where we introduced algae and the other half where we added only growth media. Significance tests for those comparisons were two-tailed because the ideal free distribution predicts equal fitnesses.

We reasoned that:

1. The absence of co-varying densities between the habitats would rule out density-dependent habitat selection in favour of diffusion.
2. An ideal free distribution would be achieved if the density in one habitat depended on that in the other, if the pattern of density mirrored that predicted from the control habitats, and if there was no difference in mean fitness between them.
3. *Chlamydomonas* is capable of adaptive habitat selection, but incapable of equalizing fitness, if density in one habitat depended on that in the other, if mean fitness was higher in the light habitat, and if cells preferentially moved to or remained in the light.

RESULTS

Diffusion

Mean optical densities of media with dye were significantly higher in the initial than alternate side of the Petri dishes for a few hours following inoculation. Concentrations were virtually indistinguishable from one another after 5 h of diffusion (evolutionary-ecology.com/data/2869Appendix.pdf; *Tests for diffusion*). This result confirms our assumption that the growth media and any accompanying cells would diffuse throughout the Petri dishes well before the end of the habitat-selection phase in our experiments.

Habitat selection in unused media

Per capita population growth rate was higher in the control light than in the control shade (Fig. 3). Growth rates declined with population density linearly, and in parallel, in both habitats (equations A and B in Table 1, Fig. 3).

To determine whether the habitat-selection experiments yielded an ideal free distribution, we set equations (A) and (B) in Table 1 equal to one another:

$$2.37 - 1.20 \times (\text{Density in light}) = 1.87 - 1.29 \times (\text{Density in shade}) \quad (6)$$

and solved for the habitat isodar with reference to the density of cells occupying the light habitat. Thus, for an ideal free distribution,

$$\text{Density in light} = 0.42 + 1.08 \times \text{Density in shade} \quad (7)$$

Table 1. Relationships of per capita population growth rates (fitness) and cell densities with habitat (geometric mean regression; 95% confidence intervals in parentheses)

Comparison	Regression equation	F	d.f.	P	Eqn.
Per capita population growth rate and density (controls)	$W_{light} = 2.37 (\pm 0.2) - 1.20 (\pm 0.3) \times N_{light}$	44.0	1, 28	<0.001	A
	$W_{shade} = 1.87 (\pm 0.2) - 1.29 (\pm 0.4) \times N_{shade}$	6.4	1, 28	0.02	B
Density between sides (controls)	$N_{Alternate\ light} = -0.10 (\pm 0.3) + 0.82 (\pm 0.4) \times N_{Initial\ light}$	9.3	1, 13	0.01	C
	$N_{Alternate\ shade} = -0.02 (\pm 0.2) + 0.96 (\pm 0.4) \times N_{Initial\ shade}$	8.7	1, 13	0.01	D
Density between habitats	$N_{Alternate\ shade} = -0.03 (\pm 0.1) + 0.54 (\pm 0.2) \times N_{Initial\ light}$	21.5	1, 13	<0.001	E
	$N_{Alternate\ light} = -0.03 (\pm 0.2) + 1.05 (\pm 0.4) \times N_{Initial\ shade}$	26.6	1, 13	<0.001	F

Note: Comparisons based on experiments enabling *Chlamydomonas reinhardtii* to choose between two sides of Petri dishes containing freshly prepared growth media. All regressions were statistically significant (**bold** font). Quadratic regressions yielded a competing model for per capita population growth in control light (AICc Linear = 11.83; AICc Quadratic = 11.6; simpler linear model chosen because it had fewer parameters) but not for control shade (AICc Linear = 23.89; AICc Quadratic = 26.16).

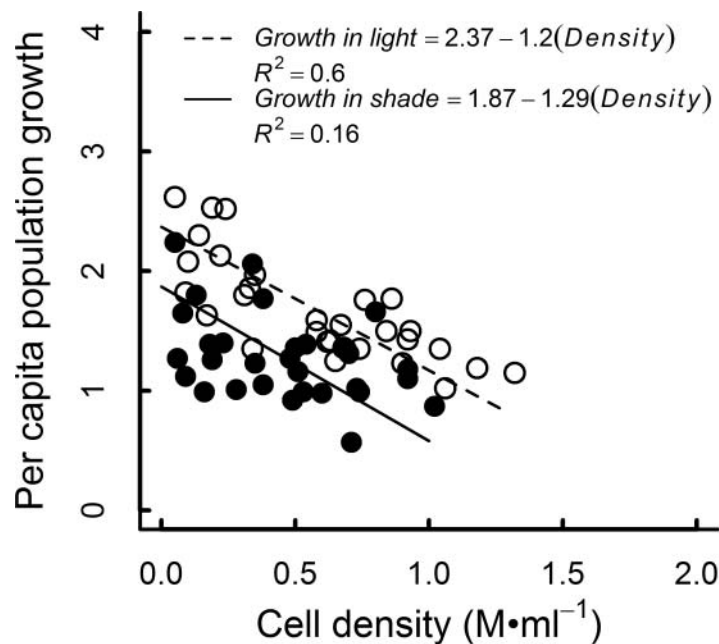


Fig. 3. The relationships between per capita population growth rates (fitness) and *Chlamydomonas* cell density (millions of cells per millilitre) in the control light (open circles) and control shade (solid circles) habitats with unused media ($n = 30$ for each regression).

(dashed line, Figs. 4c, d). According to the isodar, cells should occupy only the light habitat at densities below 0.42 million cells per millilitre, then become ever more evenly distributed between habitats with increasing population size. The same outcome occurs when habitat selection is devalued by diffusion. In both cases, however, the intercept will be reduced if the reduction in fitness by resisting diffusion exceeds that of acclimation (equation 4).

All regressions comparing cell densities between sides and habitats in unused media were statistically significant (equations C–F, Table 1). Intercepts were not different from zero and slopes were not different from one in all comparisons of density between sides of control dishes (Table 1). Similarly, there was no difference in the mean per capita population growth rates between initial and alternate sides of the control Petri dishes (Table 2). Cells given a choice between the two identical halves of the control dishes followed an ideal free distribution.

When released in light, there was no difference in mean fitness of *Chlamydomonas* between light and shade habitats (Table 2), but the resulting isodar departed from that predicted from the control dishes (intercept not different from zero, slope larger than unity; Fig. 4c). Cells did not achieve an ideal distribution when released in the shaded half of the habitat-selection dishes. Although there was no difference in density between habitats (Table 1, Fig. 4d), fitness was higher in the light habitat (Table 2).

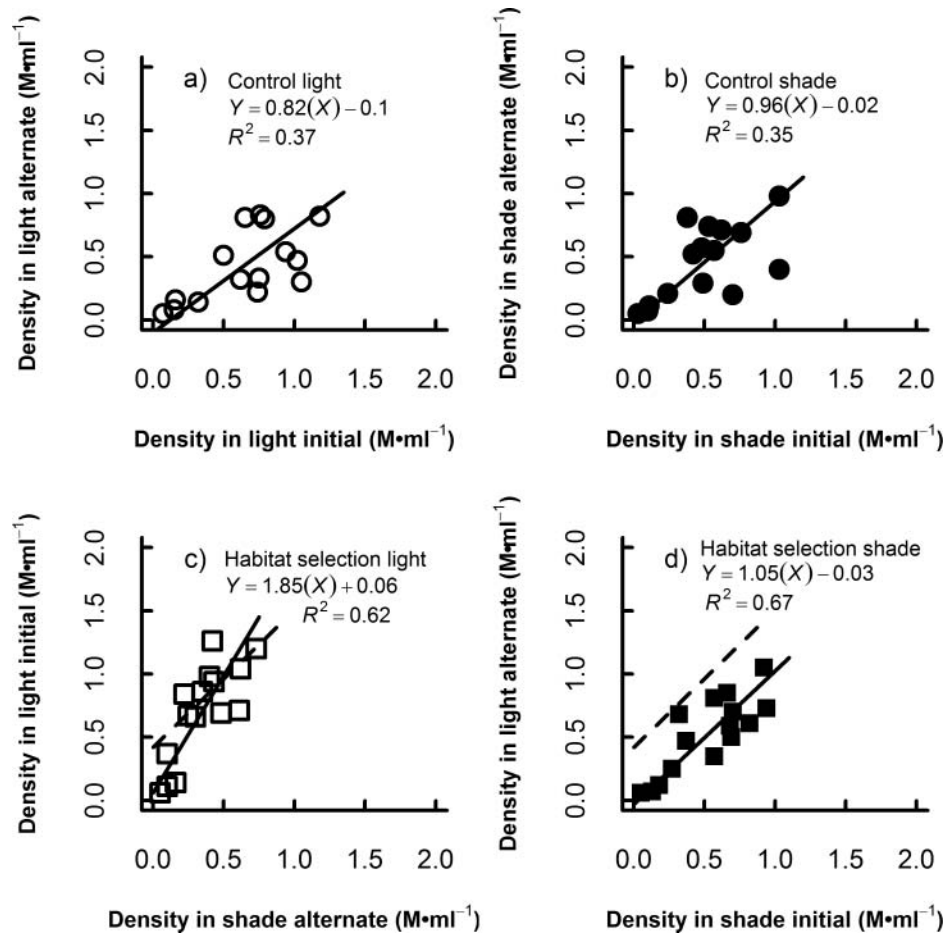


Fig. 4. The 'isodar graphs' of *Chlamydomonas reinhardtii* (millions of cells per millilitre) living in unused media. (a, b) Habitat isodars comparing initial and alternate sides of control dishes. (c, d) Regressions of density from the treatment dishes. Open data points represent experiments initiated in the light habitat, solid data points correspond to experiments initiated in the shade. Dashed lines represent the ideal free isodar predicted from comparisons between shade and light control dishes.

Habitat selection in used media

Again, as in unused media, per capita population growth rate was higher in the control light than in the control shade (Fig. 5). Growth rates in controls declined with population density linearly, but much more slowly in shade than in light (equations G and H in Table 3, Fig. 5). The maximum per capita population growth in the control light was slightly lower than in unused media, as was the decline with density (Table 3; contrast Fig. 3 with Fig. 5). Maximum per capita growth in shade, on the other hand, was much less than in unused media, and the decline in fitness was far more gradual. There was no difference in fitness between paired halves of control dishes in light (Table 5), but the regression comparing paired densities in the two sides of the dishes was not significant (Fig. 6a, no evidence for density-dependent habitat selection). Fitness and density in the initial shade side exceeded

Table 2. Comparisons of *Chlamydomonas* per capita population growth rates between the initial and alternate sides of Petri dishes containing controls and habitat-selection treatments with freshly prepared growth media

Experiment	Side	Habitat	Mean fitness	Paired <i>t</i> -test	<i>P</i>
Control light	Initial	Light	1.56	1.92	0.08
	Alternate	Light	1.77		
Control shade	Initial	Shade	1.37	−1.32	0.21
	Alternate	Shade	1.19		
Introduced in light	Initial	Light	1.55	−1.8	0.09
	Alternate	Shade	1.35		
Introduced in shade	Initial	Shade	1.10	3.97	0.001
	Alternate	Light	1.79		

Note: **Bold** font indicates statistical significance (non-IFD). Paired *t*-tests; two-tailed significance.

that in the alternate shaded side (Table 4, Fig. 6b). Contrary to unused media, control shade cells in used media failed to equalize fitness. Nevertheless, we set equations (G) and (H) (Table 3) equal to one another,

$$2.15 - 0.82 \times (\text{Density in light}) = 1.27 - 0.39 \times (\text{Density in shade}) \quad (8)$$

and solved the expected isodar,

$$\text{Density in light} = 1.07 + 0.48 \times \text{Density in shade} \quad (9)$$

(dashed line, Figs. 6c, d). According to equation (9), algae should occupy only the light habitat at densities below 1.07 million cells per millilitre. Then, for each increase in population size, approximately half as many cells should occupy the lighted half of the dish as occupy the shaded half. The intercept should be lower than predicted if acclimation and resisting diffusion reduce fitness.

Neither habitat-selection treatment with used media produced patterns in habitat densities consistent with the predicted isodar. Densities in the two habitats fit linear one-to-one relationships with zero intercepts and slopes of unity (Table 3, Figs. 6c, d). Per capita population growth rates were significantly greater in light regardless of which habitat the cells were introduced into (Table 4). Despite no clear evidence of differences in density between habitats, the pattern in the residuals suggests a possible preference for the light habitat at low density (Figs. 6c, d). This preference, if real, is consistent with the higher per capita population growth rates observed in that habitat at low density (Fig. 5).

DISCUSSION

Habitat selection operates through the process of adaptive movement such that individuals can increase fitness by moving to a different habitat. Organisms should thus evolve motile and sensory capabilities that enable preferential occupation of habitats yielding higher fitness than others. The experiments reported here document that even simple organisms are capable of adaptive movement that produce repeated, but often less than ideal, patterns of habitat selection.

Table 3. Relationships of per capita population growth rates (fitness) and cell densities with habitat (geometric mean regression; 95% confidence intervals in parentheses)

Comparison	Regression equation	F	d.f.	P	Eqn.
Per capita population growth rate and density (controls)	$W_{light} = 2.15 (\pm 0.2) - 0.82 (\pm 0.2) \times N_{light}$	32.6	1, 25	<0.001	G
	$W_{shade} = 1.27 (\pm 0.1) - 0.39 (\pm 0.1) \times N_{shade}$	13.2	1, 27	0.001	H
Density between sides (controls)	$N_{Alternate\ light} = 0.12 (\pm 0.3) + 0.67 (\pm 0.4) \times N_{Initial\ light}$	3.5	1, 13	0.08	I
	$N_{Alternate\ shade} = -0.06 (\pm 0.2) + 1.32 (\pm 0.4) \times N_{Initial\ shade}$	9.6	1, 13	<0.01	J
Density between habitats	$N_{Alternate\ shade} = -0.12 (\pm 0.2) + 0.91 (\pm 0.4) \times N_{Initial\ light}$	14.2	1, 13	<0.01	K
	$N_{Alternate\ light} = -0.15 (\pm 0.2) + 0.84 (\pm 0.4) \times N_{Initial\ shade}$	7.4	1, 13	0.02	L

Note: Comparisons based on experiments enabling *Chlamydomonas reinhardtii* to choose between two sides of Petri dishes containing used growth media. **Bold** font indicates statistical significance. Quadratic regressions yielded a competing model for per capita population growth in control light (AICc Linear = -1.51; AICc Quadratic = -0.23, simpler linear model chosen because it had fewer parameters) but not for control shade (AICc Linear = -36.05; AICc Quadratic = -33.35).

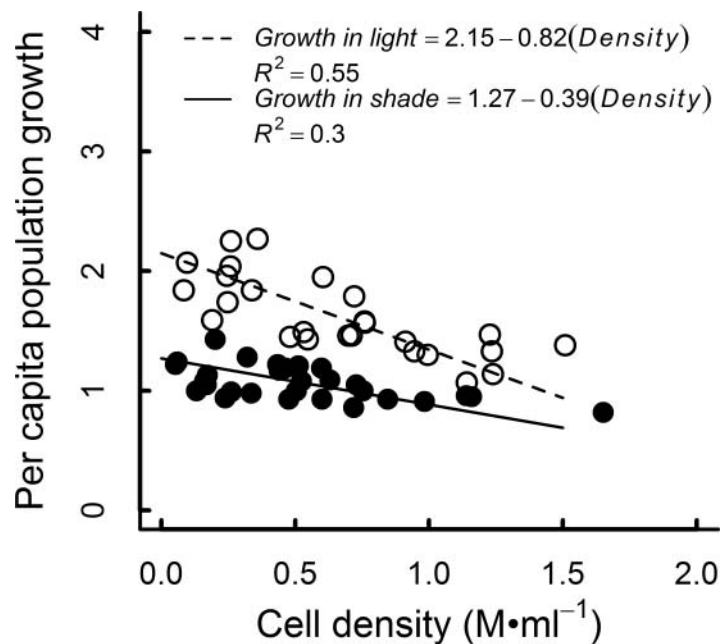


Fig. 5. The relationships between fitness (per capita population growth rates) and *Chlamydomonas* cell density (millions of cells per millilitre) in the control light (open circles) and control shade (solid circles) habitats with used media ($n = 26$ and $n = 29$ respectively).

Adaptive habitat selection was best demonstrated in the experiments with unused media. When cells were released in either light or shade controls, there was no difference in density or fitness between the two halves of the dishes. Cells moving between identical 'habitats' do not change photosystems and swim through the media with normal flagellar movements that are unlikely to entail significant additional costs of habitat selection. Our data and design cannot distinguish whether this 'ideal free' result was caused by phototaxis or random diffusion throughout the Petri dish. But when cells were introduced into the light habitat and given the opportunity to move to shade, density remained greater in light even though there was sufficient time to equilibrate densities between habitats. Cells released in light thus attained an ideal distribution such that fitness was not different between habitats. Densities, however, were not those predicted from the controls.

In contrast, there was no detectable difference in density between habitats when cells were exposed to used media. The similar densities were maintained even though mean fitness was higher in the light than in the shade in all treatments where cells could 'choose' between them. Habitat selection was more 'turgid' when both sides of the Petri dishes contained used media.

The distributions of cells between habitats appear to confirm the prediction that organisms are most capable of adaptive movement when signals of habitat quality are distinct and reliable. Random diffusion appeared to equalize cell densities when the signal was ambiguous. But diffusing populations retained the differential population growth associated with each habitat. We speculate that the differences in growth rates reflect a signal of habitat quality below the stimulus that cells require to preferentially occupy the light

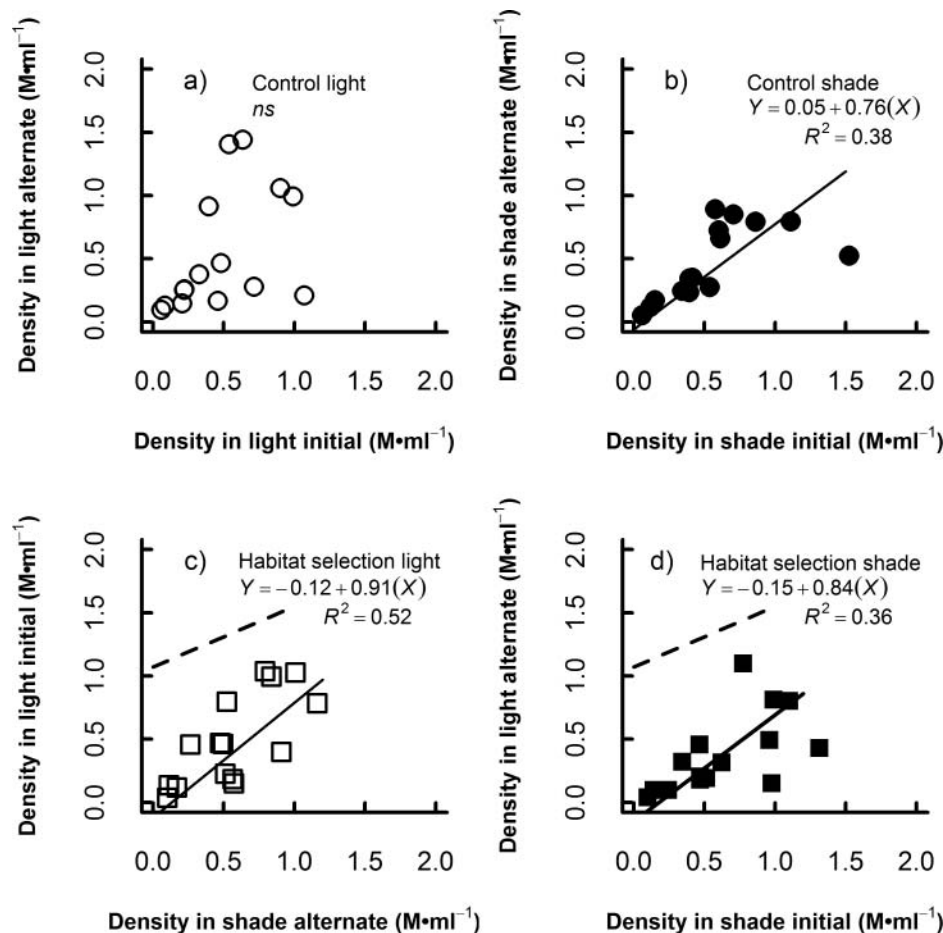


Fig. 6. The ‘isodar graphs’ of *Chlamydomonas reinhardtii* (millions of cells per millilitre) living in used media. (a, b) Habitat isodars comparing initial and alternate sides of control dishes. (c, d) Regressions of density from the treatment dishes. Open data points represent experiments initiated in the light habitat, solid data points correspond to experiments initiated in the shade. Dashed lines represent the ideal free isodar predicted from comparisons between shade and light control dishes.

habitat. If so, then our research answers Robertson and colleagues’ (2013) call for experiments that manipulate fitness benefits in order to measure cue thresholds associated with adaptive behaviours.

We do not assume that cells are capable of assessing fitness directly. Rather, we imagine that cues of habitat quality indicate, with error, a threshold beyond which the cells switch from diffusion to active phototaxis. Cells receiving habitat stimuli below the threshold will simply diffuse through the medium. Cells receiving habitat stimuli above the threshold become phototactic towards the better habitat. Perceptual errors associated with ambiguous signals thus appear to cause the algae to undervalue (Gilroy and Sutherland, 2007), and consequently undermatch (e.g. Abrahams, 1983; Sutherland *et al.*, 1988; Kennedy and Gray, 1993), the light habitat. The inability of shade controls to equalize fitness in used media suggests that

Table 4. Comparisons of *Chlamydomonas* per capita population growth rates between the initial and alternate sides of Petri dishes containing controls and habitat-selection treatments with used media

Experiment	Side	Habitat	Mean fitness	Paired <i>t</i> -test	<i>P</i>
Control light	Initial	Light	1.56	1.82	0.09
	Alternate	Light	1.91		
Control shade	Initial	Shade	1.12	−2.20	0.05
	Alternate	Shade	1.04		
Introduced in light	Initial	Light	1.81	−5.01	<0.001
	Alternate	Shade	1.04		
Introduced in shade	Initial	Shade	1.12	3.97	<0.001
	Alternate	Light	2.16		

Note: **Bold** font indicates statistical significance (non-IFD). Paired *t*-tests; two-tailed significance.

patterns in density are further complicated by an apparent interaction between habitat quality and the rate of diffusion.

Readers may recognize a paradox in our interpretation. Cells released in light with unused media occupied that habitat preferentially only at high densities. We can suggest two possible reasons for this anomaly. First, the isodar's zero intercept could simply indicate that the fitness cost of resisting diffusion exceeds that of acclimation (e.g. equation 3). Second, there may be unaccounted costs associated with living in light at low density. Cells living at high light intensities are exposed to increased oxidation of photosystems that require expensive cellular repairs (Harris *et al.*, 2009). It is thus possible that the costs of oxidative stress in light at low cell densities, when shading from other cells is minimal, limit the otherwise density-dependent benefits associated with occupying that habitat. Such an effect would imply that signal detection and phototactic responses to light vary with light intensity and, indirectly, with density (density-dependent habitat selection).

There is a third possibility. Cells entering the shade by chance or design pay an acclimation cost that they may be unable to reclaim by returning to light. Even so, cells in shade achieve reasonably high fitness at low density and may thus possess a higher threshold for movement than at high density. Future developments of choice models based on signal detection should include the effect of density.

Readers may also question whether there is a second paradox. We assumed that the experiments with used media represented lower-quality habitat. But the mean fitness of cells living in light was similar between the two types of media. There are two explanations for this pattern. First, the fitness of control-light cells growing in unused media declined more rapidly than that of control-light cells in used media (cf. Figs. 3 and 5). Per capita resource competition was thus more intense in the richer environment. Second, densities in used media were biased towards shade habitat in the habitat-selection experiments (cf. Figs. 4c, d with Figs. 6c, d). Cells were at lower density in the light habitat in these experiments than they were in unused media, and thus attained higher mean fitness than might otherwise have been expected.

We also do not assume that the cells were adapted to the specific range and types of cues provided in our experiments. We assume, instead, that *C. reinhardtii* acquire adaptations such as sensitivity to light and nutrients through much longer periods of selection. We

similarly assume that cells undergoing such evolution will nevertheless evolve towards some threshold response where they alter their behaviour to remain in a habitat, move to another one, or diffuse randomly throughout their environment.

Regardless as to mechanisms, selection of high fitness habitat by *C. reinhardtii* in this study was conditional on whether nutrient concentrations were high (unused media) or low (used media). Although conditional strategies of habitat selection are inferior to density-dependent habitat choice, they can be adaptive when habitats of different quality remain constant over long periods of time (Morris *et al.*, 2004). It will be interesting to learn whether habitat selection by more sentient organisms also depends on differences between habitats conditioned by density dependence and the mean quality of the environment.

Adaptive movement emphasizes the benefits of increased fitness as individuals select habitat in response to differences in their environment (Abrams, 2000; Cressman and Krivan, 2013). The distribution and fitness of *C. reinhardtii* in our experiments documents abilities of adaptive habitat choice originally developed mainly for sentient organisms (Fretwell and Lucas, 1969; Flaxman and deRoos, 2007). Our research demonstrates rather clearly that even so-called simple single-celled organisms are capable of adaptive movements that modify spatial distribution and population dynamics. These movements, despite numerous contingencies and interactions, are nevertheless consistent with theories of habitat selection that link density-dependent habitat choice with population regulation and species interactions (Rosenzweig, 1981; Morris, 1988, 2003). The biggest surprise, however, is not that algae are capable of adaptive habitat selection, but rather that adaptive movement is so often neglected by ecologists studying the dynamics of populations and communities.

ACKNOWLEDGEMENTS

We thank M. Maki and S. Schroeder for their assistance in the laboratory, G. Bell for suggesting *Chlamydomonas reinhardtii* as a model for habitat selection, and A. Dupuch, W. Halliday, S. Vijayan, R. Buchkowski, S. Hecnar, and B. Danielson for insightful advice and comments. We are indebted to M. Rosenzweig for several typically sage suggestions that improved the quality and value of this contribution. We are thankful for the continuing support of D. Morris's research programme in evolutionary ecology provided by Canada's Natural Sciences and Engineering Research Council (NSERC), and for the opportunity to conduct this research in Lakehead University's Biorefining Research Institute.

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