

Habitat availability mediates chironomid density-dependent oviposition

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Abstract Knowledge of density-dependent processes and how they are mediated by environmental factors is critically important for understanding population and community ecology of insects, as well as for mitigating harmful insect-borne diseases. Here, we tested whether the oviposition of chironomids (Diptera: Chironomidae; non-biting midges), known to carry the Cholera pathogen *Vibrio cholerae*, is density dependent and if it is mediated by habitat availability. We used two multiple choice

experiments in habitat-limited and habitat-unlimited environments and performed isodar analysis on counts of egg batches after controlling the polarization of light reflected from the habitats, which is known to affect their attractiveness to ovipositing chironomids. We found that, when habitats are limited, egg batch isodars indicate that chironomid selection is density dependent. Although a greater number of individuals selected to oviposit in highly polarized sites, oviposition was also common in sites with low polarization. When habitats are unlimited, chironomid selection is either weakly density dependent, or completely density independent. Chironomids oviposit to a very large extent in sites with high level of polarization, oviposit to a small extent in sites with medium level of polarization, and almost completely disregard unpolarized sites. We suggest that ovipositing females consider the availability of habitats in their surroundings when they choose an oviposition site. When high quality habitats are scarce, more females opt to breed in low quality sites. These findings may be used to limit the spread of Cholera by controlling the habitats available for chironomid oviposition.

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Introduction

Animals are frequently distributed unevenly between natural habitats. This distribution often results from the uneven distribution of food resources in their environment and from the nature of the competition for these limited resources (Fretwell and Lucas 1969; Rosenzweig 1985). The density of individuals may negatively affect their food

intake rate, in many cases due to the increase in food resource consumption by conspecifics (Lotka 1925; Volterra 1926; Gause 1934; Fretwell and Lucas 1969; Charnov 1976). Where more than one suitable habitat is available and individuals are free to move between habitats with negligible cost, animals in a food-rich and highly dense habitat are expected to constantly compare their per capita food intake with the option of inhabiting an alternative food-poor but less dense habitat. Consequently, animal density in the habitat richer in food is expected to be higher than that of the habitat poorer in food (Fretwell and Lucas 1969; Charnov 1976; Stephens and Krebs 1986; Morris 1987). This uneven distribution has been termed the “ideal free distribution” (IFD), as it is an ideal (optimal) distribution of individuals that are free to move between habitats (Fretwell and Lucas 1969). When the overall number of individuals increases, animal distribution in the two habitats is expected to match resource density (Parker 1978), thus reflecting the suitability of the habitats in supporting an increasing number of individuals (Morris 1987, 1988, 1994). An optimal forager making habitat selection decisions should therefore take into consideration the density of competitors at each site (Rosenzweig 1985). Competitor density may be particularly critical during periods of reproduction, when habitat selection decisions such as where to breed and forage have consequences on the offspring.

Density-dependent consequences are known in mammals (e.g., gerbils; Ovadia and Abramsky 1995), birds (e.g., eagles; Ferrer and Donazar 1996), reptiles (e.g., lizards; Calsbeek 2009), amphibians (e.g., frogs; Crump 1991), fish (e.g., sculpin; Petty and Grossman 2010), mollusca (e.g., snails; Kawata et al. 2005), and insects (e.g., fleas; Hawlena et al. 2005). However, the response of the animals to conspecific density may differ markedly in different taxa. The presence of conspecific eggs may deter an ovipositing female due to the expected future competition (Resetarits and Wilbur 1989). In contrast, it may boost oviposition when it is perceived as an indicator of habitat quality or because of “group” or “dilution” effects (Dia-Eldin and Ward 1991; Edgerly et al. 1998; Rudolf and Rodel 2005) that may lower the risk of predation of any single offspring. Another factor that may affect habitat selection decisions is the availability of suitable habitats. Increasing the number of suitable habitats for reproduction in a given area is expected to decrease individual density in any specific habitat, since it reduces competition for resources. Yet, empirical evidence supporting this prediction is rare. Female mosquitoes were found to respond to an increase in habitat availability by increasing their overall egg clutches laid but decreasing the number of egg clutches laid in any single habitat (Reiskind and Wilson 2004),

presumably in order to lower their offsprings’ future competition from conspecifics while increasing their overall reproductive output.

Different animal densities may result from the habitat selection decisions of individuals, but may also be the outcome of demographic parameters (e.g., survival and recruitment) that differ among different habitats (Morris 1988). However, when multiple choices are offered to animals from the same population, demographic processes can be ruled out and density dependence or density independence can be deduced from measurements of animal density. Furthermore, differences in animal density may inform us about the way animal distribution is regulated by animal density. One of the methods to study such effects is isodar analysis, which was developed to investigate density-dependent effects on habitat selection, population dynamics, and community structure (Morris 1987, 1988, 1994, 1999; Ovadia and Abramsky 1995; Shochat et al. 2002). In isodar analysis, animal density in a high quality (usually food-rich) habitat is plotted against animal density in a low quality (often food-poor) habitat, and a regression line (the isodar line) is fit to the data. The isodar line reflects the suitability–density functions (SDF) of the two habitats, which describe how the quality of each habitat decreases with increasing population density (Morris 1987, 1988).

Since the isodar line represents the ratio of individuals in the different habitats, their preferences and whether their distribution depends on population density can be interpreted from the slope and the intercept of the isodar (Morris 1987, 1988; Shochat et al. 2002). The intercept of the isodar informs us about the initial preference of the animals when their population density is relatively low, while the slope of the isodar informs us about the animals’ selection when the number of individuals increases. A slope of one indicates that a new animal entering the area has an equal probability of selecting one of the two habitats, while a slope significantly higher than one indicates that one of the two habitats is preferred (the higher the slope, the higher the preference). An intercept of zero suggests that there is no preference for either of the two habitats at low densities, while an intercept greater than zero suggests that, at low densities, the animals occupy only one of the two habitats and, from some threshold density, they also start using the other habitat. Density-dependent habitat selection can be deduced from isodars with an intercept greater than zero and/or a slope greater than one (Morris 1988). Note that density-independent habitat selection (reflected by an infinite or zero isodar slope) is indicated when no individuals occupy one of the habitats, regardless of the density in the other habitat (Morris 1987, 1988).

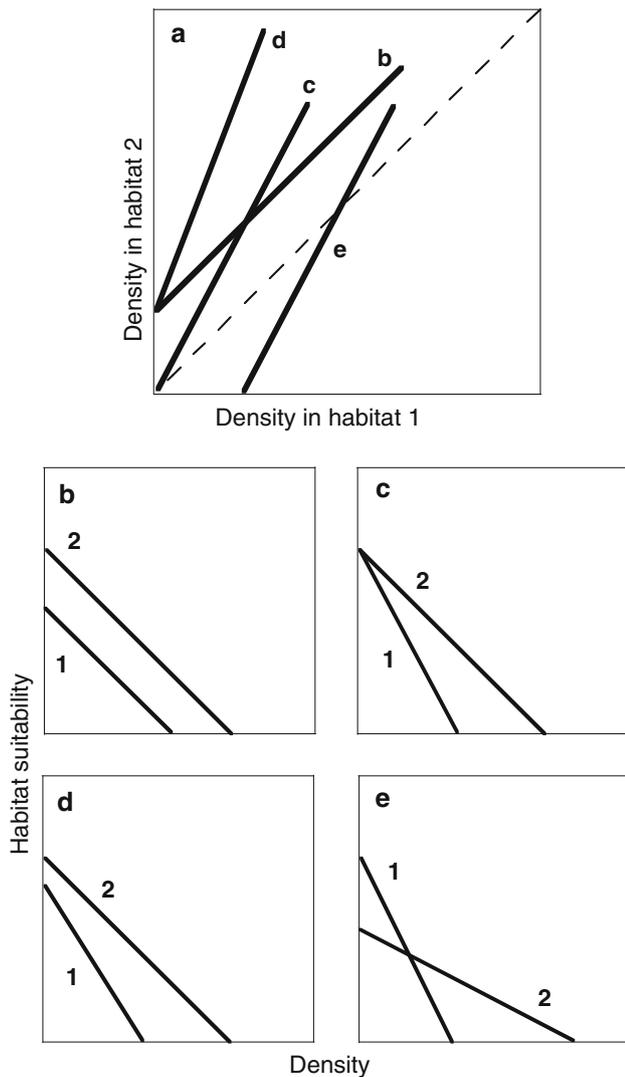


Fig. 1 Isodar relationships and corresponding habitat suitability-density functions (SDFs). **a** Isodars representing different combinations of slope and intercept values, each labeled with its corresponding SDF panel. The *dashed line* represents the $y = x$ line. SDFs include: *b* intercept >0 and slope = 1, *c* intercept = 0 and slope >1 , *d* intercept >0 and slope >1 , and *e* intercept <0 and slope >1 . In **b–e**, SDFs for habitats 1 and 2 are represented by 1 and 2, respectively. Note that in all the cases presented here, animal distribution is density dependent

Following Morris (1988), we have illustrated in Fig. 1a several possible scenarios of density-dependent habitat selection:

1. An isodar with a slope = 1 and an intercept >0 (Fig. 1b). In this case, at low population density, the individuals occupy only the rich habitat until its suitability declines and become comparable to the other habitat. Then, at higher population density, the rate of resource depletion with increasing population density is equal in the two habitats, and thus they are

selected by equal numbers of newly arriving individuals. The slope of the SDF of the two habitats is equal, but one habitat is richer than the other (has a higher suitability at any given density), representing a case of parallel regulation of animal population.

2. An isodar with a slope >1 and an intercept = 0 (Fig. 1c). In this case, there is no difference in the preference of the individuals for either one of the two habitats at low population density, but with increasing population density, the rate of resource depletion is lower in the rich habitat, and a greater proportion of individuals selects the rich habitat. Under this scenario, the SDF of the two habitats exhibits a diverging pattern, with a shared starting point representing their equal suitability under low population density.
3. An isodar with a slope >1 and an intercept >0 (Fig. 1d). In this case, at low population density, the individuals occupy only the rich habitat. With increasing population density, the rate of resource depletion is lower in the rich habitat, and a greater proportion of individuals continues to select the rich habitat. Under this scenario, the SDF of the two habitats again exhibits a diverging pattern, but with the richer habitat having a higher suitability even at low population densities.
4. An isodar with a slope >1 and an intercept <0 (Fig. 1e). In this case, at low population density, the individuals occupy only one of the two habitats. With increasing population density, when the animals also enter the second habitat, the rate of resource depletion in the first habitat is greater than in the newly occupied habitat, and consequently a greater proportion of individuals enters the second habitat. Under this scenario the SDF of the two habitats cross one another, with one habitat having a higher suitability at low population densities and the other having higher suitability at high population densities.

To detect the different scenarios, one needs to plot the matched densities of the individuals in each pair of habitats and to infer about the habitat distribution relationship from the fitted model (Morris 1987, 1988, 1994, 1999; see “Materials and methods”).

We used isodar analysis to study the oviposition behavior of chironomids (Diptera: Chironomidae; non-biting midges). Chironomids are globally distributed freshwater insects, and their adults live for only several hours. They mate in mid-air around sunset and die soon after without feeding. After mating, each female descends to lay only one egg batch (EB), containing hundreds of eggs glued together in a gelatinous sac, attaching the EB to the surface of a water body, such as pond, puddle, or lake. About 70% of the females lay their EB at dusk, while the

rest wait until dawn to oviposit (Armitage et al. 1995). Chironomids were found to host and carry the *Vibrio cholerae* bacterium, which is responsible for the fatal Cholera disease in humans (Broza and Halpern 2001; Halpern et al. 2004; Broza et al. 2005, 2008). It is suspected that, during oviposition, the bacteria are transferred to the water via the EB's gelatinous sac. Ovipositing patterns may therefore impact the spread of the disease.

Chironomid oviposition site selection has been found to correlate with several biotic factors, such as conspecific and predator density, as well as with abiotic factors, such as habitat moisture and temperature (Rudolf and Rodel 2005). It is presumed that these factors inform the laying females about the prospects of the habitat for the survival and development of their offspring. One species of chironomid, *Chironomus oppositus*, was found to avoid water bodies containing the freshwater gastropod *Physa acuta* by using visual and odor cues (Devereaux and Mokany 2006), while another species, *C. tepperi*, was found to avoid water bodies containing conspecifics as well as the larvae of other chironomid species (Stevens et al. 2003).

It has recently been discovered that *C. transvaalensis* females are inclined to oviposit in water bodies reflecting a high percentage of linearly polarized light, while disregarding the intensity of the reflection and the chemical attributes (odor) of these water bodies (Lerner et al. 2008; Meltser et al. 2008). The attraction of female chironomids to polarized water bodies is presumably advantageous due to the positive relationship between reflected polarization and organic carbon concentration in the water on which chironomid larvae feed (Lerner et al. 2008). The percent polarization of the reflected light can therefore serve as a reliable cue for ovipositing females seeking food-rich habitats for their young. However, chironomid larvae may consequently face intense competition in highly polarized sites, which may result in low larva food intake rate, reduced larva survival, and impaired fitness of the ovipositing female. To mitigate such hazardous risks, female chironomids may select to oviposit in habitats characterized by lower conspecific egg density, for example in habitats characterized by low reflected polarization level, when their preferred habitats are highly dense with conspecific EB.

In the present study, we investigated if chironomid oviposition site selection is density dependent, and how habitat availability may mediate density-dependent chironomid oviposition. We apply isodar analysis to chironomid oviposition count data from two experiments in which habitat availability was either limited or unlimited to check (1) if chironomid oviposition is density dependent (according to isodar slope and/or intercept), and (2) if changing habitat availability affects habitat selection

(according to differences in isodar slope and/or intercept when habitat availability varies). We expect to find evidence of both density dependence in chironomid oviposition and variations in chironomid habitat selection due to varying habitat availability.

Materials and methods

Experimental design

Two multiple choice experiments were carried out on the banks of a waste stabilization pond (60 × 70 m) near Tiv'on, Israel (32°40'N, 35°07'E), where a large natural chironomid population thrives. In the first experiment (Experiment 1), conducted under artificial illumination in a habitat-limited environment (a closed tent), 10 repeats of the same experiment were conducted, one on each of 10 nights during fall 2007. The females were lured by a fluorescent light to enter a tent positioned on the banks of the pond at sunset. After trapping the females inside the tent, it was sealed, the fluorescent light was turned off, and a set of four egg traps was introduced to the chironomids. Each trap consisted of a 30 × 30 × 30 cm wooden box, on the bottom of which was a 10 W bulb. Above the bulb, there was a 10 × 10 cm open hatch with a 15 × 15 cm filter. The filter included varying numbers of diffusers and a plane polarizer that provided polarized/unpolarized illumination at two levels of intensity, by using filters with 3 and 15 diffusers. By flipping the filters, the four egg traps illuminated high/low intensity ($2.0/0.5 \times 10^{-5} \mu\text{E cm}^{-2} \text{s}^{-1}$) of polarized/unpolarized light (>95/<5%) (PLIL, low polarization low intensity; PLIH, low polarization high intensity; PHIL, high polarization low intensity; PHIH, high polarization high intensity). A 15 × 15 × 10 cm glass aquarium filled with 2 cm (450 cm³) of tap water was placed on each filter and served as the egg laying site ("egg trap"). Egg traps were placed at sunset of each sampling day and were left overnight, during which time the chironomid adults mated and the females oviposited. On the following morning of each sampling night, EB were counted, and the traps were cleaned and prepared for the following night. The location of the different treatments within the set was randomized from night to night.

In the second experiment (Experiment 2), conducted under natural illumination in a habitat-unlimited environment (outdoors, on the banks of the pond where chironomids regularly oviposit), sets of four tubs that served as egg traps were positioned next to each other and placed about 5 m from the edge of the water surface. As in Experiment 1, the egg traps remained for an entire night, and EB were

collected in the following morning. Each tub ($40 \times 50 \times 20$ cm; 40 L) was filled with 20 L of water. The tubs were placed for four sampling nights during fall 2007, at four stations, located on each of the four banks of the pond, totaling 16 sets (repeats). As in Experiment 1, in each set, the four tubs reflected different levels of light intensity and polarization. This was achieved by painting two of the black tubs with white paint up to the water level and by filling the tubs with tap or pond water (WT, white painted tub with tap water; WP, white painted tub with pond water; BT, black unpainted tub with tap water; BP, black unpainted tub with pond water). Note that the smell of the white paint did not affect chironomid oviposition behavior, since chironomid females do not oviposit according to odor (Meltser et al. 2008). WT reflected light with very low percent polarization ($4.6 \pm 2.1\%$, $n = 10$ different measurements), WP reflected light with a medium level of percent polarization ($20.4 \pm 8.3\%$, $n = 10$), while the light reflected from BT and BP was relatively highly polarized (35.4 ± 7.0 , $n = 18$, and $38.5 \pm 12.0\%$, $n = 18$, respectively). The intensity of the reflected light from WT traps was $2.06 \pm 0.07 \times 10^{-3} \mu\text{E cm}^{-2} \text{s}^{-1} \text{nm}^{-1}$, an order of magnitude higher than in the other three traps (1.37 ± 0.04 , 1.53 ± 0.06 , and $1.86 \pm 0.06 \times 10^{-4} \mu\text{E cm}^{-2} \text{s}^{-1} \text{nm}^{-1}$). The full details of these measurements are described in Lerner et al. (2008). We note that the tubs' water edge, where the EB were attached, was not physically limited (i.e., there was much more room for more females to oviposit) in all the treatments during the two experiments, even when oviposition was the densest.

Isodar analysis

To test for density-dependent habitat selection, we plotted isodar lines using EB counts from pairs of habitats (treatments) (Morris 1987, 1988, 1994). We determined for any pair of treatments examined which is the habitat that is regarded as "poor" and which is the habitat regarded as "rich" by the level of intensity and polarization of the reflected light in the habitats, following the findings of Lerner et al. (2008). Each isodar was created by plotting the number of EB found in the rich habitat's traps against the number of EB found in the poor habitat's traps, so that the data from each repeat in the "poor" and "rich" habitats created a single x - y point. The isodar data collected over the different nights, with 10 repeats of Experiment 1 and 16 repeats of Experiment 2, allowed us to use linear regression analysis in the density plane, where the x -axis is the density of EB found in the "poor" habitat, and the y -axis is the density of EB found in the "rich" habitat. We used geometric mean regression (also known as 'reduced' major axis type-II regression) analysis using a Matlab code provided by Peltzer (2010) to fit each isodar line. This

particular regression model was selected based on its unbiased calculations of regression statistics compared with other regression analyses (Kermack and Haldane 1950; Ricker 1973). To calculate the confidence intervals of the slope and the intercept, we applied the formula provided by Sokal and Rohlf (1995) with $\alpha = 0.05$. We infer about chironomid habitat preference and density dependence during oviposition according to the isodar slope and intercept calculated by this regression analysis (see Fig. 1).

Results

Chironomid oviposition in the two experiments

In Experiment 1, carried out inside the tent in habitat-limited conditions, EB of four chironomid species were found, of which 83% were laid by *Chironomus transvaalensis*. In Experiment 2, carried out outdoors in habitat-unlimited conditions, only *C. transvaalensis* EB were found in the traps. Totals of 10,592 EB were counted during Experiment 1 and 3,543 EB during Experiment 2. Average (\pm SD) EB counts in each treatment are reported in Table 1 (see Lerner et al. 2008 for raw count data). Under the habitat-limited conditions of Experiment 1, a significantly nonzero number of EB were laid in the unpolarized traps (PLIL) (95% confidence interval $\text{CI}_{95} = 54.0\text{--}241.2$ EB repeat $^{-1}$), while under the habitat-unlimited conditions of Experiment 2, the number of EB laid in the unpolarized WT traps was not statistically different from zero ($\text{CI}_{95} = -0.2$ to 2.8 EB repeat $^{-1}$).

Table 1 The number of chironomid egg batches (EB) in the different treatments of Experiment 1 and Experiment 2

Treatment	Polarization (%)	Intensity ^a	EB counts (mean \pm SD)
Experiment 1 (habitat-limited)			
PLIL	<5	Low	105.2 \pm 74.5
PLIH	<5	High	271.8 \pm 133.1
PHIL	>95	Low	268.8 \pm 210.2
PHIH	>95	High	413.4 \pm 199.1
Experiment 2 (habitat-unlimited)			
WT	4.6 polarized	High	0.8 \pm 1.3
WP	20.4 polarized	Low	11.1 \pm 11.7
BT	35.4 polarized	Low	93.9 \pm 114.6
BP	38.5 polarized	Low	115.6 \pm 118.8

PLIH Low polarization and high intensity, PLIL low polarization and low intensity, PHIL high polarization and low intensity, PHIH high polarization and high intensity, WT white painted tub with tap water, WP white painted with pond water, BT black unpainted tub with tap water, BP black unpainted tub with pond water

^a Low/high intensities = $0.5/2.0 \times 10^{-5} \mu\text{E cm}^{-2} \text{s}^{-1}$

Isodar analysis of chironomid oviposition

Under the habitat-limited conditions of Experiment 1, the isodar slope is significantly greater than one and the intercept greater than zero in the PLIH–PLIL isodar (Fig. 2a), the isodar slope equals one and the intercept is greater than zero in the PHIH–PHIL isodar (Fig. 2b), and the isodar slope equals one and the intercept is greater than zero in the PHIL–PLIL isodar (Fig. 2c), and the isodar slope is greater than one and the intercept not

significantly different from zero in the PHIH–PLIH isodar (Fig. 2d; Table 2). These three cases suggest that, when the number of habitats is limited, chironomid habitat selection is density dependent. Chironomids prefer rich habitats, but there is still substantial oviposition in habitats that are regarded as poor based on their low reflected light intensity or polarization. Furthermore, they suggest that the rich habitat, characterized by higher reflected intensity or

Fig. 2 Selected isodar relationships of the number of egg batches (EB) laid in Experiment 1 in a habitat-limited environment in rich (vertical axis) and poor (horizontal axis) habitats under controlled illumination ($n = 10$). The different treatments are: *PLIH* extremely low polarization and high intensity reflecting patch; *PLIL* extremely low polarization and low intensity; *PHIL* high polarization and low intensity; and *PHIH* high polarization and high intensity. Full statistical details of the regressions are provided in Table 2

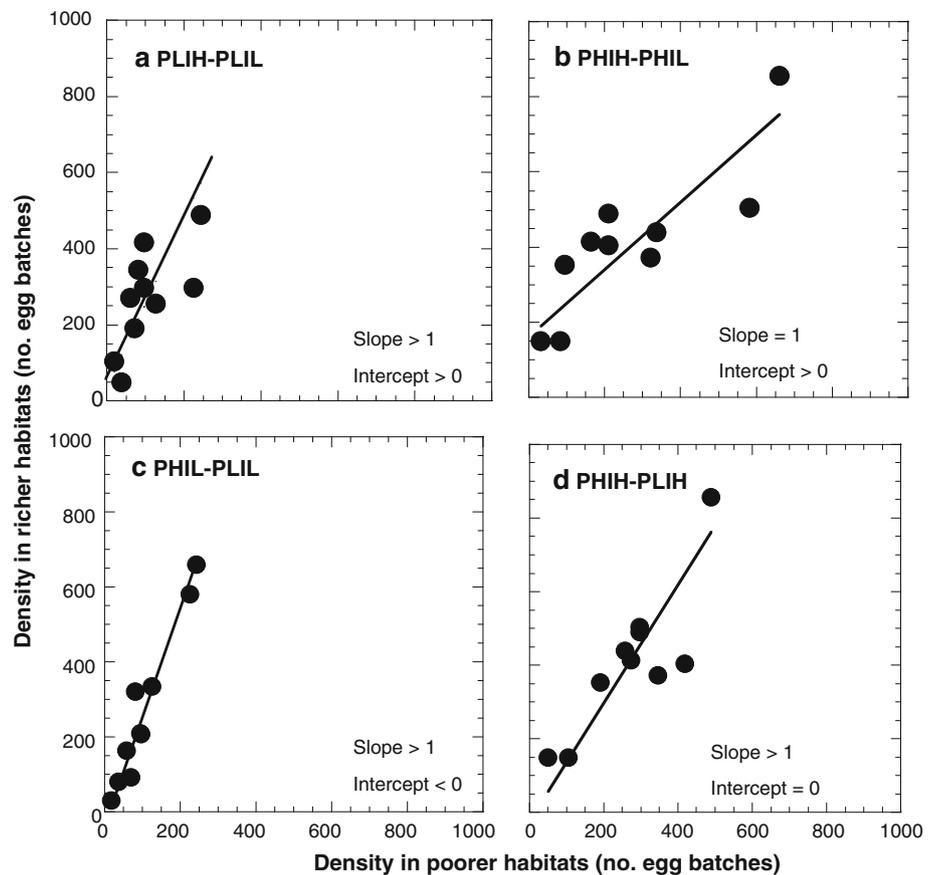


Table 2 Chironomid isodars found by geometric mean regressions on egg batch (EB) counts in different treatment pairs in each experiment (see Table 1 for treatment abbreviations)

Egg traps examined (rich vs. poor)	Figure	Model r^2 (P value)	Slope		Intercept	
			Mean \pm SD	95% Confidence intervals	Mean \pm SD	95% Confidence intervals
Experiment 1						
PLIH vs. PLIL	2a	0.49 (0.02)	1.8 \pm 0.5	1.4–2.2	83.9 \pm 58.5	41.2 to 126.6
PHIH vs. PHIL	2b	0.72 (0.001)	0.9 \pm 0.2	0.8–1.0	158.8 \pm 57.0	117.2 to 200.4
PHIL vs. PLIL	2c	0.94 (0.0001)	2.8 \pm 0.2	2.7–2.9	–28.0 \pm 31.2	–50.8 to –5.2
PHIH vs. PLIH	2d	0.72 (0.0009)	1.5 \pm 0.3	1.4–1.7	6.8 \pm 79.7	–51.3 to 64.9
Experiment 2						
BT vs. WP	3a	0.55 (0.0007)	9.8 \pm 1.9	8.8–10.8	–14.7 \pm 29.9	–30.7 to 1.3
BP vs. WP	3b	0.58 (0.0004)	10.2 \pm 1.9	9.2–11.2	2.95 \pm 29.8	–13.0 to 18.9
BT vs. BP	3c	0.94 (0.001)	1.0 \pm 0.1	0.9–1.1	18.2 \pm 9.0	13.4 to 23.0

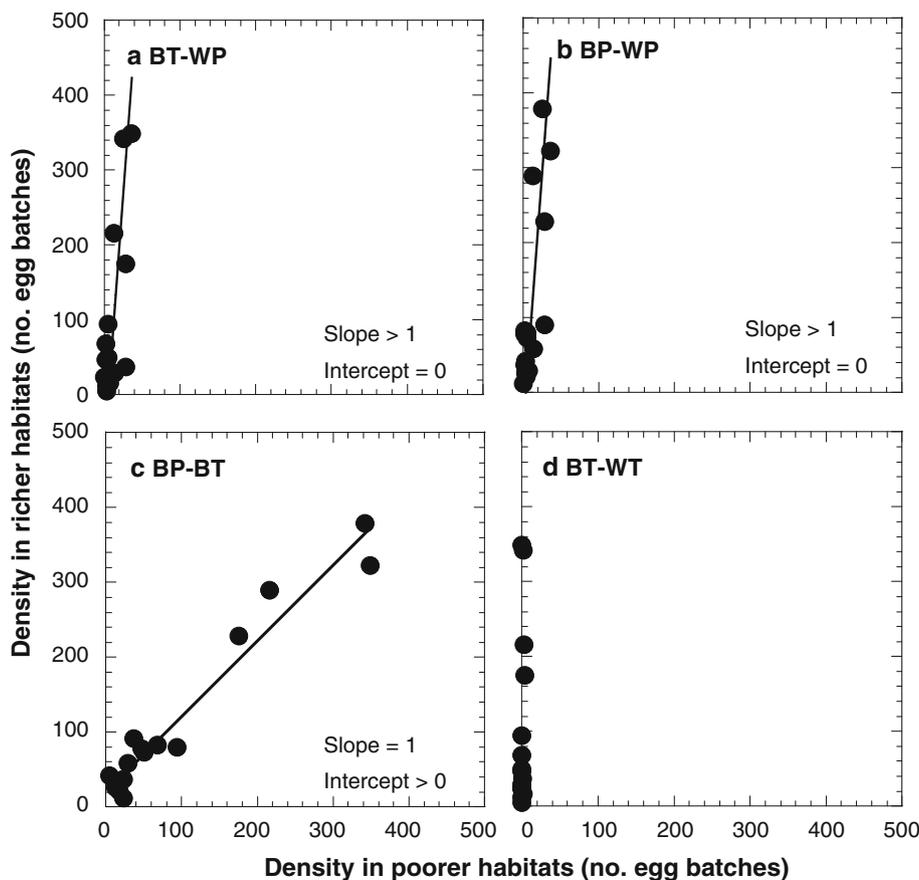
polarization, has a higher suitability than the poor one (see Fig. 1). The highly positive slope and the slightly, yet significant, negative intercept of the fourth isodar, PHIL–PLIL (Table 2) also suggests that chironomid habitat selection is density dependent under habitat-limited conditions, but that the shape of the suitability–density function of those two treatments is of crossover regulation (see Fig. 1); under low population density, the animals prefer the habitat characterized by a low level of reflected polarization, while at higher population densities, the animals prefer the habitat characterized by a high level of reflected polarization.

Under the habitat-unlimited conditions of Experiment 2, the BT–WP and the BP–WP isodars had a slope greater than one and an intercept not different from zero, suggesting that, when habitat is unlimited, habitat selection is also density dependent (Fig. 3a, b; Table 2), and that the corresponding SDFs diverge (see Fig. 1). The slopes of the two isodars (≈ 10) are much steeper than the slopes of the isodars in the habitat-limited conditions of Experiment 1 (1.5–2.8), suggesting that chironomids are much more selective outdoors and oviposit less in habitats that are perceived as sub-optimal based on their lower reflected polarization. This indicates that, under such scenarios,

chironomid density-dependent habitat selection is weak. When count data from the two habitats with similar, highly polarized reflection are plotted against each other (BT–BP), the isodar slope is not significantly different from one while the intercept is significantly greater than zero (Fig. 3c; Table 2), suggesting SDFs of parallel regulation (see Fig. 1). Yet, the absolute value of this difference in EB between BT and BP is relatively small (≈ 18 EB) given the range of densities (up to 379 EB) sampled in these habitats, and this can be explained by the very small difference in percent polarization between these two treatments (3.1%; Table 1).

We could not calculate isodar statistics for BT–WT and BP–WT treatment pairs because it is a prerequisite that population size varies between the different samples in the density plane such that the data will contain samples of population density at low and high population sizes (Morris 1987, p. 271). WT data included mostly zeros (10 out of 16 samples), and the number of EB counted in each of the non-zero samples was very small (1–4 EB). Overall, WT data suggest that female chironomids avoid ovipositing in low-polarized sites located outdoors, as opposed to when oviposition habitats were limited within the tent.

Fig. 3 Selected isodar relationships of the number of egg batches (EB) laid in Experiment 2 in a habitat-unlimited environment in rich (vertical axis) and poor (horizontal axis) habitats under natural illumination ($n = 16$). The different treatments are: WT white painted tub with tap water (lowest polarization and highest reflected light intensity of all habitats); WP white painted tub with pond water (low polarization); BT black unpainted tub with tap water (high polarization); BP black unpainted tub with pond water (high polarization). Full statistical details of the regressions are provided in Table 2



Discussion

Our results suggest that: (1) chironomid oviposition is not made on a random basis. The pattern of the SDF in the different habitats follows differences in the level of reflected polarization, with higher suitability in sites characterized by a higher level of polarization throughout the range of population densities sampled; (2) chironomid oviposition is not restricted to the most suitable (rich) habitat since chironomids often use sub-optimal habitats in a density-dependent manner; and (3) chironomid oviposition is markedly affected by habitat availability through a change in female selectivity. When habitat availability is unlimited, a smaller share of the females' reproductive efforts is allocated to moderately polarized, sub-optimal, sites, while unpolarized sites are completely avoided.

A negative response to conspecific offspring has been described in the behavior of laying female treefrogs. For example, in the Cope's grey treefrog *Hyla chrysoscelis*, fewer females laid less eggs per visit in habitats containing conspecific tadpoles compared with control habitats without conspecific tadpoles (Resetarits and Wilbur 1989). These authors proposed that females perceive the presence of conspecific tadpoles as a potential threat of future competition to their offspring rather than as a cue for the habitat's high quality or as a means to lower the offsprings' predation risk by aggregation (Edgerly et al. 1998). In the Günther's Costa Rican treefrog *Hyla pseudopuma*, a switch from a highly preferred habitat (tubs with deep water) to a less preferable habitat (tubs with shallow water) was documented when conspecific egg number increased in the preferred habitat (Crump 1991). Our findings suggest that laying chironomids perceive the presence of conspecifics in oviposition habitats similarly to treefrogs, and consider the expected future competition of their larvae when selecting among different habitats.

The main species investigated in the present study (*C. transvaalensis*) is a short-lived organism, and hence the females have a strong driving force to oviposit as soon as they mate, during the night. Consequently, these organisms may assess the availability of oviposition sites in the limited area that can be explored during the mating period that lasts several hours. Under such circumstances, habitat availability near the mating area is probably critical for chironomid reproduction. The fact that egg density was substantially lower in the habitat-unlimited experiment versus the habitat-limited experiment is in line with the findings of Reiskind and Wilson (2004), who studied mosquito *Culex restuans* oviposition and similarly showed that when more habitats are available for oviposition, the density of larvae in each habitat decreases.

Isodar analysis is a powerful method to quantify the effects of competition on animal distribution among different habitats (Morris 1987, 1988, 1994, 1999). Yet, one has to bear in mind that certain errors in the process of estimating the isodar line may result in findings that are difficult to interpret. For example, in the PHIL–PLIL isodar (Fig. 3c), the intercept is significantly negative, while the slope is larger than one (Table 2). According to theory, such a case may indicate a crossover regulation of animal density in the two habitats, with one habitat having a higher suitability at low population densities, and the other habitat having a higher suitability at high population densities (Morris 1987, 1988; Fig. 1e). Since in all cases, including low population density, EB counts are higher in the habitat with highly polarized reflection compared with counts in the low polarized habitat, we suggest that the negative intercept found in this isodar is caused by errors in the statistical estimation of the isodar line, specifically in the estimation of the intercept near the origin. Such spurious effects do not provide reliable information about the animals' actual preferences at low densities because no shift in density-dependent behavior (i.e., preference for food-poorer habitat when densities are low) occurred. This isodar estimation error may potentially be resolved by increasing data sampling. A similar case was reported by Shochat et al. (2005) in one of their studied species, the red-winged blackbird (*Agelaius phoeniceus*), in which isodar statistics suggested a behavioral shift between preferred habitats with increasing density, but no actual shift occurred.

Isodar estimation may also depend on the specific model used, and there are three different isodar models that are relevant to the study of habitat selection (Morris 1994). The first model, named the “continuous input model”, assumes that resources in the habitats are continuously consumed by the individuals immediately after they become available for consumption. It is characterized by a linear isodar line on a log–log scale (log density in habitat 1 vs. log density in habitat 2). The second model does not assume an immediate consumption of resources by the individuals and is named the “consumer–resource model”. This model is characterized by a linear isodar line on a linear–linear scale (density in habitat 1 against density in habitat 2). The third model, which is called the “pre-emptive model”, defines the quality of the habitats by their abundance of discrete breeding sites (e.g., holes that are suitable sites for nest-hole species) and is characterized by nonlinear isodar relations. Since, in our experiments, each artificial habitat was considered a single, non-fragmented, breeding habitat without any discrete within-habitat breeding sites, and since food resources were not immediately consumed by foraging animals, we applied the

“consumer–resource model” to describe chironomid oviposition behavior. However, unlike earlier studies that used this model (e.g., Morris 1988; Shochat et al. 2002), in the present study, density dependence was assessed by laying females considering that their offspring will live the consequences of their oviposition habitat selection decisions. Therefore, our findings propose a novel inter-generational version of the “consumer–resource model”.

Chironomid oviposition may have consequences on the infection and spread of Cholera (Broza and Halpern 2001; Halpern et al. 2004; Broza et al. 2005, 2008). We previously suggested that minimizing chironomid oviposition in drinking water reservoirs can be achieved by manipulating the level of polarization reflected from their surface (Lerner et al. 2008). Here, we propose that minimizing chironomid oviposition in drinking water resources can be further improved by a combination of measures. Besides making drinking water sources physically unfavorable to chironomids by decreasing their reflected polarization, providing many other attractive (highly polarized), alternative oviposition sites nearby may alter chironomid oviposition away from the drinking water source. To reduce density-dependent effects of conspecific eggs that may deter chironomid females from laying in these alternative habitats, constant removal of the eggs must be undertaken. Using these simple measures to control chironomid oviposition may reduce the infection and spread of Cholera in countries where it poses a serious threat to public health.

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