Published in final edited form as:

Ecol Entomol. 2019 April ; 44(2): 197–205. doi:10.1111/een.12689.

Effects of larval density on a natural population of *Culex restuans* (Diptera: Culicidae): No evidence of compensatory mortality

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Abstract

1. We investigated the effects of strong density-dependence on larval growth, development, and survival of the mosquito *Culex restuans* (Theobald). We tested the hypothesis that density reduction early in larval development could result in as many or more individuals surviving to adulthood (compensation or overcompensation, respectively), or increased reproductive performance via rapid development and greater adult size.
2. In a field study of a natural population of *C. restuans* we tested for the effects of a 75% lower density on percent survivorship to adulthood, number of adults, development time, adult size, adult longevity, and size dependent fecundity.
3. We found no evidence for compensation or overcompensation in adult production, nor for effects of lower density on percent survivorship. Low density yielded significant increases in adult size, adult longevity, and size-dependent fecundity, and a decrease in development time.
4. Estimated per capita population growth rate was significantly greater in the low-density treatment than in the high-density treatment. We infer this difference resulted from greater per capita resources increasing female size and fecundity, and reducing development time. Greater per capita population growth could therefore result from early mortality of larvae, meaning that the hydra effect, which predicts greater equilibrium population with, as opposed to without, extrinsic mortality, may be possible for these mosquitoes.

Graphical Abstract

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Contribution of Authors

G.D.O conceived the ideas with input from S.A.J. G.D.O collected the data. G.D.O. and S.A.J. analysed the data. G.D.O wrote the first draft of the manuscript, and S.A.J and G.D.O. revised the manuscript.

Conflicts of Interest

None declared.

- With a 75% density reduction in 1st instar *Culex restuans*, there was no evidence of compensation or overcompensation in adult mosquito production.
- Reduced density resulted in significant increases in *C. restuans* adult size, adult longevity, size-dependent fecundity, and decreased development time.
- The estimated per capita growth rate was significantly greater in the low-density treatment likely due to increased female size and fecundity, and reduced development time.

Keywords

hydra effect; density-dependence; compensatory mortality; overcompensatory mortality

Introduction

When a population that is regulated by density-dependence (e.g., from limited food resources) is perturbed by an extrinsic source of mortality (e.g., predation) it is possible that the number of survivors may be unaffected by, or increase with, increased mortality. This paradoxical effect may occur because extrinsic mortality releases the population from the stronger effect of intraspecific competition, with the result that the same number of individuals survive (compensatory mortality), or possibly even a greater number of individuals survive (overcompensatory mortality) than would be the case without that extrinsic mortality (Washburn *et al.*, 1991; Juliano, 2007; Abrams, 2009). Abrams and Matsuda (2005) noted that an increase in average population density in response to increased mortality can be an outcome of overcompensation, and termed this the “hydra effect” (after the mythological creature that grew two new heads for each head severed). A variety of models postulating different mechanisms predict that overcompensatory mortality may produce the hydra effect, which may be widespread in nature, but empirical evidence for its extent and importance is limited (de Roos *et al.*, 2007; Abrams, 2009).

Determining the conditions under which the hydra effect occurs in nature is of practical importance for both conserving populations harvested by humans (Anderson & Burnham, 1976; Pedersen *et al.*, 2004; Péron, 2013), and for control of invasive species (e.g., garlic mustard; Pardini *et al.*, 2009) and vectors of disease (Juliano, 2007). If wildlife managers set inflated harvest quotas based on the assumption that hunting mortality is compensatory when it is actually additive, it could lead to overexploitation, collapse and even extinction of the harvested species (Sale & Tolimieri, 2000; Pedersen *et al.*, 2004; Pöysä, 2004; Péron, 2013). Conversely, when the management goal is controlling an invasive species or disease vector that is under density-dependent regulation, the hydra effect could cause the undesirable outcome of the target species increasing, rather than decreasing in population size (Zipkin *et al.*, 2009).

Mosquitoes are frequent targets of control because they are important vectors of arboviruses (Eldridge *et al.*, 2004). *Culex restuans* (Theobald, 1901) is widespread in the United States (Eldridge *et al.*, 1972; Darsie & Ward, 2005) with larvae that develop in ground pools and water-filled containers. *Culex restuans* infected with West Nile virus have been found in the wild (Centers for Disease Control and Prevention, 2000), and they are capable of transmitting the virus (Sardelis *et al.*, 2001). *Culex restuans* adult females likely feed primarily on birds (Sardelis *et al.*, 2001; Molaei *et al.*, 2006). Due to their high abundance in the early spring, *C. restuans* has been postulated to be the zoonotic vector overwintering and amplifying West Nile virus (Andreadis *et al.*, 2001; Sardelis *et al.*, 2001) and St. Louis encephalitis virus (Mitchell *et al.*, 1980; Reiter, 1988). Controlling *C. restuans* populations could, in principle, reduce the likelihood of infection with these arboviruses for bridge vector mosquitoes (e.g., *C. pipiens*), which bite both birds and mammals later in the season. *Culex restuans* is virtually equivalent as a competitor to *C. pipiens* and these species often co-occur in the same aquatic habitats (Reiskind & Wilson, 2008). Thus, *C. restuans* serves as a useful model system for understanding the general responses of *Culex* species to larval density.

Container mosquitoes are habitat specialists, living as larvae in water-holding vessels (e.g., tree holes, cemetery vases). Container mosquitoes are likely to be under strong density-dependent regulation mostly due to intra- and interspecific competition for limited food resources (Service, 1985) and a buildup of toxic metabolic waste from overcrowding (Moore & Fisher, 1969). Any mosquito control method that results in a low density of larvae prior to density-dependent regulation could potentially result in compensation or overcompensation. The relatively short generation times and high fecundity of mosquitoes increases their potential to rebound quickly from extrinsic perturbation, increasing the likelihood that compensation or overcompensation will occur (Péron, 2013).

The primary objective of this research was to quantify how density-dependence influences the quantity and quality of adult mosquitoes produced in a field population of *C. restuans*. The hypothesis of overcompensation predicts that containers in which density is low will produce more adult mosquitoes, and that those adults, produced at low density, will be of greater quality (e.g., greater adult size and longevity, better flight performance, shorter gonotrophic cycles) compared to containers in which density is high. Larger adult females from the low-density conditions are further predicted to have greater fecundity. Because

females require more resources to reach pupation successfully, we expect the difference between high- and low-density treatments to be most pronounced for females. The hypothesis of overcompensation was tested by simulating the impact of an early-acting extrinsic source of mortality by creating a low density of newly hatched larvae in containers that is 75% less than that in high-density containers. We then compared the resulting quantity and quality of adult mosquitoes produced between the low- and high-density treatments. Such a reduction in number of hatchlings might result from natural enemies that prey selectively on small larvae (e.g., cyclopoid copepods: Marten, 1990), or from control approaches that induce hatching failure, such as sterile male release (e.g., Alphey *et al.*, 2010) or cytoplasmic incompatibility inducing *Wolbachia* (e.g., O'Connor *et al.*, 2012).

Materials and Methods

Field experiment

To create our experimental containers we used a randomized block design, with 40 containers (7.5 L black buckets, 23 cm diameter x 23 cm height), 20 stocked with 75 first instar larvae (=Low density) and 20 stocked with 300 first instar larvae (=High density). Containers were arranged in 20 spatial blocks of 2 containers (one of each density) in a forested area at Merwin Preserve in McLean County, Illinois (40°39'10.6"N, 88°52'14.9"W).

Container mosquito species, including *C. restuans*, develop as larvae in water-filled natural and manmade containers and feed primarily on bacteria growing on senescent leaves, grass, and animal detritus that falls into the containers. Stemflow rainwater was collected for the experiment by stapling and silicone sealing foam pipe insulation to 3 trees with a zip-tied hose connecting to a 245 L barrel (Likens & Eaton, 1970). Ratchet straps were used to fasten netting over the barrels to keep mosquitoes from laying eggs in the barrels. Prior to use, stemflow water from the 3 rain barrels was homogenized to eliminate any stemflow water source random effects, and sieved (106 µm) to remove any aquatic macroinvertebrates. Each container was filled with 3 L of stemflow water 3 g/L of timothy hay (*Phleum pratense*, Oxbow Animal Health). Free-living mosquitoes were allowed to oviposit in the containers.

Culex restuans rafts laid on the night of July 14, 2015 were isolated into 24-well plates to facilitate verification of the species for larvae from each raft using antennal hair tuft position (Darsie & Ward, 2005). All of the identified larvae were mixed into a single tray and counted into 20 low-density and 20 high-density cohorts with 75 and 300 larvae, respectively, which were then added to the appropriate containers in the field. It was necessary to identify the *Culex* larvae as 1st instars because compensatory mortality is most likely when mortality acts early, before the action of density-dependence (Abrams, 2009; McIntire & Juliano, 2018). Identification of 1st instars is difficult; however we believe it is very unlikely that individuals of *Culex pipiens*, which is the species most likely to be confused with *C. restuans*, were included in the experiment. *Culex pipiens* typically is rare in late-spring and early-summer, when our experiment was conducted (Lee & Rowley, 2000; Jackson & Paulson, 2006; Lampman *et al.*, 2006). Further, a field experiment (Grech & Juliano, 2017) done in the same wooded area between July 9–19, 2015 yielded 634 *Culex* egg rafts, and 100% of resulting larvae reared to 4th instar for identification were *C. restuans*.

(Grech & Juliano, 2017). Our field site was located in a forested, rural area, and *C. restuans* is typically more abundant in rural areas than *C. pipiens* (Ebel *et al.*, 2005). The competitive abilities of *C. restuans* and *C. pipiens* are virtually identical (Reiskind & Wilson, 2008), so even in the unlikely event that some *C. pipiens* were inadvertently included, this inclusion would be unlikely to affect density dependence.

To remove any *Aedes* eggs from experimental containers, the containers were thoroughly scrubbed with bleach and rinsed with water. Each container received 3 L of stemflow water and 3 g/L of timothy hay. The timothy hay was contained in nylon mesh bags (~14 × 3 cm with 0.25 mm openings) sealed shut with zip ties. Confining the hay in bags facilitated removal of pupae from the containers throughout the experiment. The mesh bags were weighted with plastic vials filled with rocks and water to ensure that they sank to the bottom. After the density manipulation was applied, the containers were capped with screened lids to prevent further oviposition by mosquitoes.

Pupae were removed from each container every other day, taken back to the laboratory, isolated into 15 mL glass vials with mesh netting rubber banded over the tops, and placed in an environmental chamber at 25°C on a 14:10 light:dark cycle. A random selection of individuals were included in longevity assays. Time of adult emergence was recorded, water was removed from the vials, and sex determined. Adults were provided with reverse osmosis (RO) water moistened cotton, which was rewetted daily. Vials were checked every 8 hours and the time of death for each individual was recorded as an indicator of energy reserves. Wing lengths were measured for each individual as an indicator of body size, which strongly correlates with female fecundity.

Predicting *C. restuans* fecundity from wing lengths

In mosquitoes, wing length is positively correlated with body size and fecundity (Christophers, 1960; Briegel, 1990; Nasci, 1990). To predict *C. restuans* fecundity from wing lengths, free living *C. restuans* females were trapped as they laid eggs in containers holding timothy hay infusion 1 km North of Illinois State University campus in Normal, IL in the summer of 2016. Gravid float traps were assembled by hot gluing pipe insulation foam 1 cm below the outside rim of 120 mL plastic portion cup (Supplementary Materials Figure 1). The bottom of the portion cups were cut off and replaced with netting, which provided ventilation, allowing the trap to be stealthily placed over ovipositing females without turbulence from the trapped air pocket disturbing them, and eliminated condensation from the cup walls. On the following morning the egg rafts were collected and the females were frozen for later wing removal, wing image capture under a dissecting microscope, and wing measurement using the Fiji distribution of ImageJ (Schindelin *et al.*, 2012; Schneider *et al.*, 2012). Hatched larvae were identified to species as 1st instars using the position of the antennal hair tuft (Darsie and Ward 2005). Images of egg rafts captured under a dissecting microscope and eggs were counted using the cell counter plugin (De Vos, 2010) for ImageJ. A linear regression of number of eggs laid vs. wing length (PROC REG, SAS 9.4) was done, and used to predict fecundity of *C. restuans* eclosing from the field experiment.

Estimating population rate of increase

Female wing measurements were used to estimate the population rate of increase using the composite index of performance (Livdahl & Sugihara, 1984)

$$r' = \frac{\ln\left(\frac{1}{N_0} \sum_x A_x f(\overline{w}_x)\right)}{D + \frac{\sum_x x A_x f(\overline{w}_x)}{\sum_x A_x f(\overline{w}_x)}},$$

where N_0 is the initial number of female larvae stocked in the container (assumed to be 0.5 of the initial density, which is a common assumption for investigations of population performance of mosquitoes in general and *C. restuans* in particular; Livdahl & Sugihara, 1984, Léonard & Juliano, 1995 Muturi *et al.*, 2011), A_x is the number of females eclosing from a cohort on day x , D is the average time delay between female eclosion and oviposition, which was estimated to be 12 days (Léonard & Juliano, 1995; Aspbury & Juliano, 1998), and $f(\overline{w}_x)$ is the predicted number of female eggs laid estimated from the mean wing lengths of females eclosing on day x .

Statistical analysis

Survivorship to adulthood, number of adults produced, and body size were tested with a 2-way MANOVA with male and female observations as the dependent variables, and density treatment (fixed effect) and spatial block (random effect) as independent variables (PROC GLM, SAS 9.4). The magnitudes of standardized canonical coefficients were used to assess which dependent variables contributed to the significant MANOVA effects (Scheiner, 2001). Numbers of male and female adults were log transformed to meet the assumption of homogeneity of variances. A 1:1 sex ratio at hatching was assumed for calculating survivorship, because the sex of larvae that died during development could not be determined, and 1:1 sex ratios are commonly observed in *Culex* species (Clements, 1992, Vinogradova, 2000). Estimated population rate of increase was analysed with a 2-way analyses of variance (ANOVA) with density treatment as a fixed effect and spatial block as a random effect (PROC GLM, SAS 9.4).

Time to adult eclosion and time from adult eclosion to death were analysed with Cox proportional hazards models with density treatment, wing length, and sex as fixed effects, and accounting for the effect of container using the robust sandwich covariance estimator to account for intra-container dependence (PROC PHREG, SAS 9.4). Due to wing damage, 96 individuals were dropped from the emergence analysis and 28 were dropped from adult longevity analysis, bringing the sample sizes to $n = 2867$ and $n = 1672$, respectively. Hazard ratios were estimated for the high-density treatment relative to the low-density treatment. The hazard ratio describes the “risk” of an event occurring (e.g., risk of adult emergence, risk of adult death) for an individual in the high-density treatment relative to the low-density treatment. A hazard ratio significantly greater than 1 for a categorical variable indicates an elevated risk of the event occurring, whereas a hazard ratio significantly less than 1 indicates a reduced risk. For the continuous variable of wing length, the hazard ratio quantifies the

increase (>1.0) or decrease (<1.0) in risk of the event for each 1 mm of increase of wing length.

Results

Relationship between wing length and fecundity

The slope and intercept for the linear regression of eggs laid vs. wing length (mm) were significantly different from zero (Figure 1). The best-fit linear relationship was: $f(x) = 107.05x - 181.34$.

Survivorship and number of adults produced

There was a significant difference in the number of adults produced between low density and high density treatments (Table 1A) with more female and male adults produced in the high density treatment (Figure 2A). Standardized canonical coefficients indicated a stronger contribution of number of females to the density effect, and a very small contribution of number of males (Table 1A). There was no significant difference in percent survivorship between the low- and high-density treatments (Table 1B, Figure 2B).

Emergence

Time from hatch to adult eclosion depended on both the density treatment and sex, but the interaction of density and sex was not significant (Figure 3). Both males and females had a significantly lower hazard of emergence (i.e., had longer development times) at high density than at low density (i.e., the hazard ratios were <1.0 ; Females: 0.566, 95% Wald CI = 0.460 – 0.697; Males: 0.645 95% Wald CI = 0.495 – 0.841). Males emerged earlier than females (Figure 3).

Adult longevity

Longevity of adults that were only provided with water was measured as an indicator of stored energy reserves at eclosion. Adult longevity was dependent on density treatment and sex, but the interaction of density and sex was not significant (Figure 4). Longevity also increased with adult size (Figure 4). For high-density containers, adult females had 1.97X (95% Wald CIs = 1.52 – 2.57) the hazard of death as females in the low-density containers and adult males had 2.39X (95% Wald CIs = 1.90 – 2.99) the hazard of death as males in the low-density containers (Figure 4). Each mm increase in wing length significantly decreased the hazard of death, by 0.069X (95% Wald CIs = 0.056 – 0.132) thus increasing longevity with size.

Adult size

Adult size differed significantly between the low and high density treatments with larger female and male adults in the low-density treatment (Table 1C, Figure 5). A larger standardized canonical coefficient for males indicated a stronger contribution of male size to the density effect and a lesser contribution of female size (Table 1C).

Estimated population rate of increase

The population rate of increase was estimated for each container using Livdahl and Sugihara's (1984) composite index (r'). The estimated population rate of increase was significantly greater for the low-compared to the high-density treatment (Figure 6).

Discussion

Under these experimental conditions, we found no evidence of overcompensation of *C. restuans* adult production in response to low density. There was no significant difference in proportion of larvae surviving to adulthood between low- and high-density treatments and number of individuals surviving to adulthood in the low-density treatment was directly proportional to the density (Figure 2). Low density reduced competition for limited resources, resulting in significantly larger *C. restuans* females with greater predicted fecundity (Figure 5), shorter development time, (Figure 3), and a corresponding significant increase in the estimated per capita growth rate (Figure 6). However, because significantly fewer females survived to adulthood in the low-density treatment, the increase in average population density that is expected for a population exhibiting the hydra effect is unlikely for this species under the conditions in this experiment.

Adults in the low-density treatment had significantly longer wings compared to the high density treatment, and greater wing size is positively correlated with greater body mass, longevity, and fecundity in a wide number of mosquito species (Christophers, 1960; Briegel, 1990; Nasci, 1990). Female body size is also associated with another important trait of mosquitoes: female infection rate. In *Anopheles gambiae*, intermediate-sized mosquitoes were most likely to have malaria sporozoites (Lyimo & Koella, 1992). For *Aedes aegypti* sampled during a dengue outbreak, probability of dengue infection increased significantly with wing length (Juliano *et al.*, 2014) even though smaller *Aedes aegypti* have been shown to be significantly more likely to acquire dengue infection and disseminated infection when given an infectious blood meal in the laboratory (Alto *et al.*, 2008a, 2008b). Thus, low density is likely to affect multiple important traits of mosquitoes via its effect on adult size.

Males from the high-density treatment were smaller than males from the low density treatment. Small male body size can increase the chance of reproductive failure in pitcher plant mosquitoes (*Wyeomyia smithii*), but among males that reproduced successfully there were no significant differences between small and large males in longevity, or number of offspring sired (Benjamin & Bradshaw, 1994). If this pattern held for *C. restuans*, males in the low density treatment would be predicted to have greater reproductive performance than those from the high density treatment due to fewer mating failures.

Low density accelerated development of both males and females (Figure 3). Altered timing of adult emergence could have important epidemiological consequences, particularly because *C. restuans* is hypothesized to play an important role in the overwintering and early season amplification of West Nile virus (Andreadis *et al.*, 2001; Sardelis *et al.*, 2001) and St. Louis encephalitis virus (Mitchell *et al.*, 1980; Reiter, 1988). Although there was only a difference of approximately 1 day in development time between the low-density and high-density treatments, this difference in development time for females seems to have

contributed to the effect of density on our demographic index r' . It is also likely that more extreme differences in densities, or instances where repeated interventions lower density, would result in still greater differences in development time.

Females from the low-density treatment lived longer as adults in the absence of sugar sources than did females from the high-density treatment, suggesting that they accrued greater energy reserves during larval development at low density. The consequences of this greater longevity may include more gonotrophic cycles, and this effect does not show up in our analysis of the demographic index r' (which assumes only one reproductive bout; Livdahl & Sugihara, 1984). We therefore might expect the demographic consequences of low density to be even greater than indicated in our results (Fig. 6). Beyond the demographic consequences, longer-lived females have a greater chance of biting multiple hosts and of living long enough for an acquired virus to complete its extrinsic incubation period, thus increasing the likelihood that they will become competent to transmit arboviruses.

Although we detected neither overcompensation of adult production nor evidence for the hydra effect in this experiment, we cannot conclude that overcompensation in response to mortality sources that lower larval density is universally absent in *C. restuans* nor in larval populations of *Culex* in general. Overcompensation requires that population densities be great enough to induce diminishing returns, so that at the greatest densities, there are fewer surviving adults than at intermediate densities (Schmitt *et al.*, 1999; Osenberg *et al.*, 2002). Our experiment used only two densities of larvae, and though they spanned a substantial range of larval numbers (25–100 hatchlings/L), it is possible that testing a greater number of densities of larvae, particularly greater densities, would detect a density range for overcompensation (Osenberg *et al.*, 2002). Observed densities of *Culex* larvae in naturally colonized man-made containers vary considerably. Westby and Juliano (2017) reported *Culex* densities in rain barrels in a forested habitat of 25 to 70 larvae/L. In that same forested area, Murrell and Juliano (2013) reported *Culex* densities in 16 L containers of approximately 16 to 140 larvae/L. In contrast, Johnson and Sukhdeo (2013) reported combined densities of *C. restuans* and *C. pipiens* as high as 318 larvae/L in 12 L containers. Our experimental densities thus appear to fall in the low- to mid-range of naturally occurring densities. Because we collected egg rafts that were deposited in our experimental containers, we believe our experimental larval densities span the range of naturally occurring larval densities at this site and this time; nevertheless, testing for overcompensation at higher larval densities and at other locations would be useful for understanding this phenomenon in *Culex*.

Beyond the potential for overcompensation in adult production, our experiment shows that this *Culex* species responds to low larval density by producing qualitatively different adults that are likely to affect demography, population dynamics, and potentially vectorial capacity. Low larval densities, as may arise from interventions to reduce mosquitoes, resulted in more rapid development, larger adult females, with greater expected fecundity and longevity. Thus, there is at least the potential for density reduction of larvae (as opposed to eradication of larvae) to produce results that are counterproductive for mosquito control.

This experiment isolates the direct effect of population density of *C. restuans*. Predation on mosquito populations has effects in addition to the direct effect of lethality; chemical cues of predation can alter prey behavior (Kesavaraju & Juliano, 2004, 2008; Kesavaraju *et al.*, 2007; Costanzo *et al.*, 2011), potentially resulting in more prudent resource usage by prey via reduced foraging behavior, which could increase the chances of the hydra effect occurring (Abrams, 2009). *Culex restuans* has been shown to respond to alarm cues of predation in a threat-sensitive manner by either fleeing or freezing (Ferrari *et al.*, 2007, 2008). The effect of *C. restuans* anti-predator behavior on resource levels has not been measured and it would be worthwhile investigating whether direct and indirect effects of predation might result in overcompensation and the hydra effect. Because the effects of density reduction on other life history traits (size, longevity, fecundity) are related to both vectorial capacity of the population and population growth, the effects of density dependence on *C. restuans* and other species of *Culex* suggest that unintended consequences of reductions of larval populations should be considered in choosing an approach to vector control.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgements

We thank M. Grech of the National University of Patagonia, San Juan Bosco, J. Goughnour of the Illinois Mathematics and Science Academy, and J. Oremus for assisting with field and laboratory work; the ParkLands Foundation and the Illinois Department of Natural Resources for allowing us to conduct field research at Merwin Nature Preserve; and Chipotle Mexican Grill for donating portion cups used for making the gravid float traps. Comments by two anonymous referees improved this manuscript. This research was funded with an R. D. Weigel Grant and Mockford-Thompson Research Fellowship to G.D.O. from the Beta Lambda Chapter of the Phi Sigma Biological Honors Society, and NIAID grants 1R15AI094322-01A1 and 1R15AI124005-01 to S.A.J.

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Figure 1.

Linear regression of wild captured *C. restuans* eggs laid vs. female wing length. Slope = 107.05 ± 19.52 S.E., $t_{57} = 5.48$, $p = 0.0117$; Intercept = -181.34 ± 69.63 S.E., $t_{57} = -2.6$, $p < 0.0001$, $R^2 = 0.35$

Figure 2.

A) Bivariate plot of number of female and male adults produced. B) Bivariate plot of proportion of initial number of male or female larvae that reached adulthood. Statistical results in Table 1A and 1B. For both panels, points are bivariate means $\pm$ SE. Within each panel, bivariate means associated with the same letter are not significantly different from one another.

Figure 3.

Cumulative probability of emergence of adults vs. time since hatching and analysis of experimental effects on probability of emergence. Males had a higher hazard of emerging than did females. Cox regression: Density: Wald $\chi^2 = 10.48$, $df = 1$, $p = 0.0012$; Sex: Wald $\chi^2 = 36.63$, $df = 1$, $p < 0.0001$; Density x sex: Wald $\chi^2 = 1.73$, $df = 1$, $p = 0.1889$; Wing length: Wald $\chi^2 = 5.55$, $df = 1$, $p = 0.0185$; Spatial block: Wald $\chi^2 = 906.89$, $df = 19$, $p < 0.0001$. See text for more details. H = High density, L = Low density, F = female, M = male.

Figure 4.

Survival plot of proportion adults alive vs. time since adult emergence and analysis of survival of adults. Cox regression: Density: Wald $\chi^2 = 56.57$, $df = 1$, $p < 0.0001$; Sex: Wald $\chi^2 = 72.04$, $df = 1$, $p < 0.0001$; Density x sex: Wald $\chi^2 = 1.70$, $df = 1$, $p = 0.1928$; Wing length: Wald $\chi^2 = 64.04$, $df = 1$, $p < 0.0001$; Spatial block: Wald $\chi^2 = 641.63$, $df = 19$, $p < 0.0001$. Censored observations were adults that escaped during longevity trials. H = High density, L = Low density, F = female, M = male.

Figure 5.

Bivariate plot of adult wing sizes for males and females. Statistical results in Table 1C.

Points are bivariate means $\pm$ SE. Bivariate means associated with the same letter are not significantly different.

Figure 6.

The estimated per capita rate of increase r' (Mean $\pm$ SE) for High and Low density treatments. ANOVA: Density: $F_{1,19} = 16.70$, $p = 0.0006$; Spatial block: $F_{19,19} = 1.43$, $p = 0.2233$. Means associated with the same letter are not significantly different.

Table 1.

MANOVAs for A) number of adults produced, B) survivorship to adulthood, and C) wing lengths.

					Standardized canonical coefficients	
Treatment	Num DF	Den DF	Pillai's Trace	P	# female adults	# male adults
<i>A) Number of female and male adults produced</i>						
Density	2	18	0.922	< 0.0001	2.618	0.033
Block	38	38	1.111	0.2471	3.460	-1.095
<i>B) Female and male survivorship</i>						
Density	2	18	0.038	0.7050	1.383	-0.386
Block	38	38	1.16	0.1620	1.378	-0.372
<i>C) Female and male wing lengths</i>						
Density	2	18	0.777	< 0.0001	0.510	1.172
Block	38	38	0.944	0.6350	-0.415	2.002