The effects of rearing density on growth, survival, and starvation resistance of the house cricket *Acheta domesticus*

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Academic editor: Ming Kai Tan | Received 12 May 2022 | Accepted 23 August 2022 | Published 21 February 2023

https://zoobank.org/AABE2159-1BCE-44F3-BA8E-C752F5FE9349

Citation: Mahavidanage S, Fuciarelli TM, Li X, Rollo CD (2023) The effects of rearing density on growth, survival, and starvation resistance of the house cricket *Acheta domesticus*. Journal of Orthoptera Research 32(1): 25–31. https://doi.org/10.3897/jor.32.86496

Abstract

Alternative food sources have become an important focus of research due to increased food demand coupled with reductions in traditional food productivity. In particular, substitutes for protein sources have been of increasing interest due to the unsustainability of traditional protein sources. Insects have been identified as a sustainable alternative to traditional protein sources, as they are easy to produce and contain essential proteins, fats, and minerals. However, mass-rearing insects requires similar considerations as farming traditional protein sources. To increase productively, growth and survival must be maximized at the highest possible densities while minimizing disease and food requirements. Here, we use the house cricket *Acheta domesticus*, a highly cultivated insect species, to investigate optimal densities for mass rearing at 14 days of age (4th instar). Nymphs were separated into density groups of 0.09, 0.19, 0.47, and 0.93 cricket/cm^2 and monitored for growth and survival. Multiple regression revealed sex ($p < 0.0001$), density ($p < 0.0001$), and sex*density interaction ($p = 0.0345$) as predictors of growth rate. Survival to maturation was significantly reduced in both 0.47 (31%) and 0.93 (45%) cricket/cm^2 groups compared to the controls. A second experiment was then conducted to investigate the starvation resistance of adult crickets reared from 14 days of age at 0.09, 0.19, 0.93, and 1.86 cricket/cm^2. A second multiple regression analysis revealed only density ($p < 0.0001$) and to a lesser extent sex ($p = 0.0005$) to be predictors of starvation resistance. These results indicate that mass-rearing house crickets is most optimal at densities < 0.93 cricket/cm^2, where impacts on survival and starvation are minimal. Although these results have implications for cricket mass rearing, research on other endpoints, including reproduction and the synergistic effects of other environmental factors, such as temperature and humidity, should be conducted.

Keywords

development, growth, insect, life history, resistance, sex differences, stress, survival

Introduction

Accelerating global population growth and consumption has put increasing strain on the world's agricultural system (Godfray et al. 2010, Sorjonen et al. 2019). Our ability to produce enough food for the world's growing population is also diminishing due to factors such as urban expansion, land degradation, climate change, and water scarcity (Sorjonen et al. 2019). As a result, levels of global undernourishment have risen to an all-time high of 9.9%, with between 720 and 811 million people facing food insecurity (United Nations 2020). Food demand worldwide is predicted to increase, with demand rising by 70% by 2070. As traditional food production methods are unlikely to fulfil current and future global food demands, alternative sources of food have been posited (Sorjonen et al. 2019).

One key area of food production that has received much attention due to its current unsustainability is traditional protein sources. Currently, global protein requirements are fulfilled largely by red meat, poultry, and seafood (Thavamani et al. 2020). These foods are a key source of several micronutrients, including iron, zinc, phosphorus, vitamin B6, and B12 (Ajwalia 2020). However, meat production is highly unsustainable, with major concerns surrounding environmental degradation, animal welfare, and the negative effects of excessive meat consumption (Thavamani et al. 2020). Along with plant-based foods, cultured meats, and mycoprotein-based foods, insect-based protein sources are growing in popularity as meat/protein alternatives (Vandeweyer 2018, Thavamani et al. 2020). Insects, like traditional protein sources, provide high volumes of fat, protein, zinc, iron, and several key vitamins (DeFoliart 1991, Schabel 2010, Alexander et al. 2017, Thavamani et al. 2020). The rearing of insects for this purpose is also more efficient and sustainable than for traditional livestock, consuming orders of magnitude less water and producing significantly less greenhouse gas emissions (GHGs) (Lundy and Parrella 2015). Insects are also superior sources of protein when compared to traditional protein products, containing a protein content of 50–82% of the dry weight (Thavamani et al. 2020). In addition to producing significantly less GHGs, insects can utilize organic waste products (low-value diets) as food sources, which provides further prospects for sustainable insect rearing (Sorjonen et al. 2020, Thavamani et al. 2020). Currently, the insect-as-food industry is expected to grow by 47% from 2019 to 2026, with 730,000 tons being produced by 2030 (Savio et al. 2022).

Despite the increasing global production of insect food sources, there is a paucity of information and research on best farming practices, including optimal densities for farmed species (Hanboonsong et al. 2013). Optimizing rearing density is one of the main considerations when seeking to increase production while maintaining food safety. Currently, it is generally understood that the effects of overcrowding include alterations in insect behavior, physiology, and, most importantly, life history (growth and survival) (Maciel-Vergara et al. 2021). However, industrial mass rearing densities are often unnaturally high, and the extent to which these densities affect insect behavior, performance, and well-being is not well understood (Francuski and Beukeboom 2020).

In industrial insect rearing, mortality is a common negative effect of increased density (Peters and Barbosa 1997). In the Mediterranean fruit fly, for example, densities of 1–15 eggs/cm^2 show increased mortality as density increases due to increased food competition (Peters and Barbosa 1997). Insect overcrowding also increases mortality as a result of increased pathogen susceptibility caused by higher temperatures, reduced immune response, and nutrient deficiencies (Vergara et al. 2021, Savio et al. 2022). Viral, fungal, bacterial, and microsporidian pathogens are all frequently found to infect mass-reared insects (Vergara et al. 2021). Viruses such as *Acheta domesticus* densovirus (AdDV) can result in mass mortality and have even been behind the bankruptcy of a few cricket-rearing companies (Weissman et al. 2012). Increases in disease transmission ultimately result in the increased mortality of mass-reared insects.

Insect growth rates are highly dependent on environmental conditions including temperature, predators, humidity, and resource availability (Barragan-Fonseca et al. 2018). Although most of these stimuli can be easily controlled, nutrient deficiencies due to often-overcrowded conditions in mass-rearing facilities can result in delayed maturation, survival, and reduced body size. These are highly negative outcomes when mass-rearing insects where high survival and increased body size are desired (Averill and Prokopy 1987, Agnew et al. 2002, Reiskind et al. 2004, Rivers and Dahlem 2014). In *Anopheles gambiae (*Jannat and Roitberg 2013), larval mortality increased by 36% in high-density groups due to waste products decreasing substrate quality. In both the fruit fly *Bactrocera tryoni* (Morimoto et al. 2019) and Western tarnished plant bugs [*Lygus hesperus* (Brent 2010)], significant reductions in adult emergence, body weight, fecundity, and energetic reserves were evident in crowded treatments. Declines in growth and prolongation of larval developmental periods have also been shown in several species, including *Culex quinquefasciatus* (Ikeshoji and Mullai 1970), *Trogoderma glabrum* (Beck 1973), *Tipula oleracea (*Laughlin 1960), *Endrosis sarcitrella (*Andersen 1956), and *Ptinus tectus (*Peters and Barbosa 1997). Increased population density can also create greater social contact and irritation, increasing competition, aggressive encounters between individuals, fighting, and habitat destruction (Southwick 1971, Weaver and Mcfarlane 1990). Mating can also be affected by overcrowded conditions (Gavrilets 2000). Martin and Hosken (2003) observed that females of the dung fly *Sepsis cynipsea* became less interested in remating when the population density increased.

Interestingly, however, some species have shown a shortening of developmental time in response to increased density (Cohen 1968, Hodjat 1969). In addition, shifts in development time and size have often been shown in both high-density (overcrowding) and low-density (group effects) treatments (Peters and Barbosa 1997). As it is often difficult to determine what rearing densities are indicative of overcrowding in any particular insect, as well as between-species behavioral and physiological differences, research on optimal densities in a variety of species is necessary. Investigating the effects of rearing densities not only on survival and growth but also on resistance to starvation due to these overcrowded conditions (i.e., resource competition) is also of interest.

Although 1900 different species of insects are consumed globally, crickets are currently one of the most highly cultivated insect species, being utilized for both human consumption and as food sources for other species (Lundy and Parrella 2015, Francuski and Beukeboom 2020, Savio et al. 2022). In this study, we investigated optimal rearing densities in the cricket *Acheta domesticus*, considering the negative outcomes of overcrowding on growth, survival, and starvation resistance in groups at densities relevant to mass insect rearing. Densities of 0.09, 0.19, 0.47, 0.93, and 1.86 cricket/cm^2 were reared starting at 14 days of age (4th instar) and maintained until death. Resistance to adult starvation was also investigated at these various densities to understand the impacts of various rearing densities on food competition, as this is likely to occur in high-density mass-rearing facilities.

Methods

Breeding colony.—House crickets *A. domesticus* were housed in a 93 × 64.2 × 46.6 cm acrylic terrarium covered with 1.5-cm thick Durofoam insulation. The colony crickets were from a homogenous stock inbred for > 60 generations. Fans on top of the enclosed structure provided air circulation. Crickets were sustained at a 12 h-day–12 h-night photoperiod and at a constant temperature of 29 °C ± 2 °C using 60-volt UV heat lamps. Ad libitum food (Country Range MultiFowl Grower, 17% protein, Quick Feeds Feed Mill, Copetown, Canada) was provided. Ad libitum distilled water was made available in soaked cellulose sponges, and egg cartons were provided for shelter. Oviposition medium (Vigoro Organic Garden Soil, The Mosaic Co., Lake Forest, IL, U.S.A.) was present in small plastic containers (7 × 7 × 7 cm). Oviposition containers were collected after a 24-hour period and incubated at 29 °C ± 2 °C until eggs hatched (~ 14 days).

Density variation.—A. domesticus were taken two weeks (14 days, 4th instar) post-hatch from a single colony oviposition container and randomly separated into four experimental groups. Crickets at this age/molt are approximately 0.25 mm in length. The experimental groups consisted of 50, 100, 250, and 500 crickets, creating densities of approximately 0.09, 0.19, 0.47, and 0.93 cricket/cm^2. respectively. Densities given are approximation. A range of densities below 0.93 cricket/cm2 was chosen to allow for density-dependent observations, as densities above this had been observed in the lab to result in mass die-offs. All groups were given a 2 × 2 egg carton, which lowered the density slightly. The 0.09 cricket/cm^2 group was chosen as the control, as the lowest-density group is expected to maximize resources per cricket and thus have fewer negative interactions. Experimental housing containers were 29 × 18.5 × 12 cm, and the crickets were housed for life and in the same conditions as the colony. Cricket containers were continuously monitored for cleanliness, as extremely dirty rearing environments may negatively impact growth and development. Containers were cleaned (crickets were moved into fresh containers) at a minimum of once a week; however, in the higher-density groups, cleaning occurred approximately every other day due to increased mortality and excrement production. Food and water were replaced daily to ensure the same resource availability in each group.

Life-history measurements.—Maturation in *A. domesticus* is denoted by the adult molt in which wings develop and individuals become sexually mature. Females are easily identifiable by the fully-developed ovipositor. Crickets reach adulthood approximately 5–60 days post-hatch. Sex, maturation mass, and development time (number of days post-hatch) were recorded for all density groups. Sample sizes for growth rate were 0.09 cricket/cm^2 (n = 41), 0.19 cricket/cm^2 (n = 68), 0.47 cricket/cm^2 (n = 142), and 0.93 cricket/cm^2 (n = 224). Sample sizes for the proportion of individuals surviving maturation were 0.09 cricket/cm^2 (n = 50), 0.19 cricket/cm^2 (n = 100), 0.47 cricket/cm^2 (n = 250), and 0.93 cricket/cm^2 (n = 500). Mass was measured using an Accuris analytical balance with a readability of 0.001 g +0.002 g. Maturation mass (g) and development time were employed to calculate the growth rate. The number of crickets that matured was used to determine the proportion of individuals that successfully matured.

Starvation resistance.—After determining the impacts of various rearing densities on growth and survival parameters, a second experiment was conducted to determine the potential responses to adult starvation at various rearing densities. Individuals from a second oviposition container were arranged as described above. Group densities were slightly altered based on our initial results that had suggested that although the proportion matured was reduced at densities ≥ 0.47 cricket/cm^2, growth rate was not affected. The experimental groups were separated and maintained as described above. Experimental groups included 50, 100, 500, and 1000 individuals, representing densities of approximately 0.09, 0.19, 0.93, and 1.86 cricket/cm^2, respectively. Three to four weeks post-maturation, 10 females and 10 males from each density group were weighed and placed in individual containers to prevent cannibalism. The mass of each individual was recorded to determine whether increased body mass is related to starvation resistance. The number of surviving individuals in the 1.86 cricket/cm^2 group consisted of 7 males and 10 females. For the starvation treatment, each individual cricket from each group was placed into a small cylindrical container and covered with plastic wrap secured by a rubber band. Holes were added to the plastic wrap to provide ventilation, and the sex and group of each individual were noted on the container. Ad libitum water was provided by placing a water-soaked cellulose sponge in the container; the sponges were re-soaked daily. Although the crickets consumed the cellulose sponges, they provided insufficient nutrients. Mortality was noted daily and used to determine longevity.

Statistics.—To determine the effect of sex, density, and possible interaction (sex*density) on both growth and starvation resistance, a multiple linear regression was conducted using the most appropriate model. The best-fit model was determined using a step-wise AICc comparison. A D'Agostino–Pearson omnibus normality test was conducted to ensure data was normally distributed. Survival curves were analyzed using a Gehan–Breslow–Wilcoxon survival analysis to determine differences in survivorship among density groups. To analyze differences in the proportion that survived to maturation, chi-square tests were applied. To determine significant differences between the various rearing densities and the control, a Fisher's exact test was applied to each rearing density compared to the control. Finally, significant differences in the mass of starvation groups were determined using a one-way ANOVA followed by Tukey's multiple comparisons test. All statistical analyses were carried out using Prism Graph Pad 9.

Results

Survival to maturation.—Chi-square tests indicated significant differences in proportion matured among the different rearing density groups ($p < 0.0001$). Fisher's exact tests indicated significant differences between the 0.47 cricket/cm^2 ($p = 0.0008$) and 0.93 cricket/cm^2 ($p < 0.0001$) groups compared to the 0.09 cricket/cm^2 density group. This constituted a 31% and 45% decrease in the proportion that matured, respectively (Fig. 1).

Growth rate.—Growth rates were collected for all rearing density groups and are reported as mean growth rate ± SD (Fig. 2). Prior to analysis, a D'Agostino-Pearson omnibus normality test was performed and confirmed normal distribution. We used AICc model selection to determine the best model for describing the relationship between sex, density, and growth. The best-fit model carried 77.46% of the cumulative model weight and included two predictors (sex and density) with interaction effects $F(3, 471) = 70.79$, $p < 0.0001$, $R^2 = 0.3108$; $y = 8.277 + 1.186\beta1 - 1.094\beta2 - 0.5763\beta3$. Results suggest that sex ($p < 0.0001$), density ($p < 0.0001$), and, to a lesser extent, sex*density ($p = 0.0345$) are significant predictors of growth rate. The multiple regression results are outlined in Table 2. A summary of growth and development measurements is outlined in Table 1.

Mass of starvation groups.—The mass (g) of each male and female *A. domesticus* used for starvation-resistant treatment was recorded immediately prior to experimentation and are represented as mean mass ± SD (Fig. 3). A one-way ANOVA indicated significant differences between groups $F(7, 69) = 58.47$, $p < 0.0001$, with a Tukey's multiple comparison test indicating significantly reduced masses ($p < 0.0001$) in both the 0.93 and 1.87 cricket/cm^2 females compared to the 0.09 cricket/cm^2 female controls. Significant reductions in mass were also detected in 0.93 ($p = 0.0018$) and 1.87 ($p < 0.0001$) cricket/cm^2 males compared to the 0.09 cricket/cm^2 male controls. Between-sex differences ($p < 0.0001$) were also detected in the 0.09, 0.19, and 0.93 cricket/cm^2 groups.

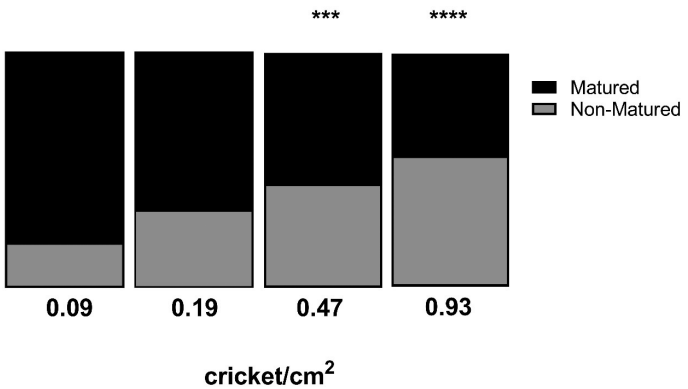

Fig. 1. The proportion of *Acheta domesticus* reaching maturation in each rearing density (0.09, 0.19, 0.47, and 0.93 cricket/cm^2). A chi-square test indicated significant differences in survival among rearing densities ($p < 0.0001$). A Fisher's exact test showed significant differences in proportion matured between 0.19 cricket/cm^2 ($p = 0.0008$) and 0.93 cricket/cm^2 ($p < 0.0001$) compared to the 0.09 cricket/cm^2 density group.

Table 1. Summary of growth parameters of each *Acheta domesticus* rearing density group.

Experimental Group	N	Growth rate (mg/day)	Percentage of controls	Upper 95% CI	Maximal growth rate	Development time (Days)	Mass at maturation (g)
0.09 Male (control)	14	7.629		8.080	8.98	50.79	0.388
0.09 Female (control)	27	8.941		9.280	10.91	48.15	0.431
0.19 Male	34	8.239	8.00%	8.518	10.00	50.00	0.413
0.19 Female	34	9.109	1.88%	9.475	12.04	48.97	0.446
0.47 Male	71	7.830	2.63%	8.026	9.68	49.51	0.387
0.47 Female	71	8.963	0.25%	9.219	11.24	47.32	0.424
0.93 Male	112	7.234	- 5.18%	7.392	9.87	48.21	0.349
0.93 Female	112	7.829	- 12.45%	8.017	10.96	46.71	0.366

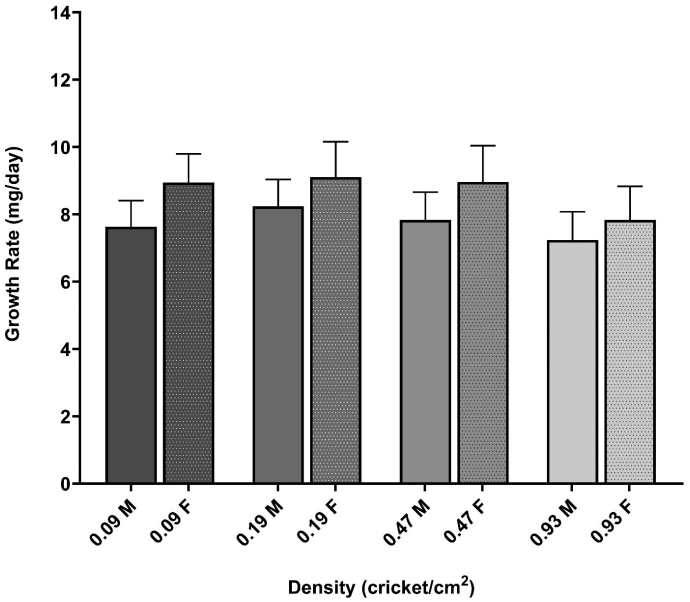

Fig. 2. Density-dependent effects of various rearing densities on the growth rate of male and female *Acheta domesticus*. Values are represented as mean growth rate of each group ± SD. Growth rates were calculated by dividing the mass at maturation (mg) by the time taken to reach maturation (days) of each individual. Multiple regression analysis determined that sex ($p < 0.0001$), density ($p < 0.0001$), and to a lesser extent sex*density ($p = 0.0345$) interaction were strong predictors of growth rate.

Density–starvation.—Starvation resistance was measured as days survived after the removal of sufficient food for all density groups and is reported as mean survival ± SD (Fig. 4). Prior to analysis, a D'Agostino–Pearson omnibus normality test confirmed normal distribution. We used AICc model selection to determine the best model to describe the relationship between sex, density, and starvation resistance. The best-fit model carried 72.65% of the cumulative model weight and included two predictor values: sex and density ($F (2, 74) = 28.05$, $p < 0.0001$, $R^2 = 0.4312$; $y = 10.46–3.661\ \beta1–2.701\beta2$). The results suggest that both sex ($p = 0.0005$) and density ($p < 0.0001$) are significant predictors of starvation resistance. Results of the multiple regression are summarized in Table 3. The Gehan–Breslow–Wilcoxon test showed significant differences in survivorship between the 0.93 cricket/cm^2 ($p = 0.0030$) and 1.86 cricket/cm^2 ($p = 0.0008$) groups compared to the lowest density (0.09 cricket/cm^2) female group. Variation in survivorship was also evident in the 0.19 ($p = 0.0328$), 0.93 ($p = 0.0063$), and 1.86 ($p = 0.0001$) cricket/cm^2 groups compared to the males in the lowest-density groups.

Table 2. Multiple linear regression analysis with AICc comparison was used to determine the most correct model for predicting growth rate based on sex and density group. AICc comparison was utilized to select the best model. Our model includes sex, density, and sex*density interactions, which carried 77.46% of the cumulative model weight. Each predictor value had a significant correlation with growth rate: sex ($p < 0.0001$), density ($p < 0.0001$), and sex*density ($p = 0.0345$).

Variable	Coefficient (β)	SE	95% CI	P Value
Intercept	8.277	0.1398	8.002 to 8.552	<0.0001
Sex	1.186	0.1885	0.815 to 1.556	<0.0001
Density	- 1.094	0.1991	- 1.485 to - 0.703	<0.0001
Sex*Density	- 0.576	0.2718	- 1.110 to - 0.042	0.0345

Table 3. Multiple linear regression analysis with AICc comparison was used to determine the most correct model for predicting survival based on sex and density group. Our model includes both sex and density, with no sex*density interactions, which carried 72.65% of the cumulative model weight. Each predictor value had a significant correlation with growth rate: sex ($p = 0.0005$), density ($p < 0.0001$).

Variable	Coefficient (β)	SE	95% CI	P Value
Intercept	10.46	0.6495	9.164 to 11.750	<0.0001
Sex	2.70	0.7451	1.217 to 4.186	0.0005
Density	- 3.66	0.5405	- 4.738 to - 2.584	<0.0001

Discussion

Insects have been proven to be a valuable alternative source of protein, fat, and essential vitamins and minerals (DeFoliart 1991, Schabel 2010, Zaelor and Kitthawee 2018). Utilizing insects as food may fill the gaps in our ability to meet current and future food demands. Crickets such as *A. domesticus* are one of the most cultivated insect species globally (Lundy and Parrella 2015, Zaelor and Kitthawee 2018, Francuski and Beukeboom 2020). *Acheta domesticus* is considered an excellent candidate for mass-rearing endeavors due to its low food requirements and beneficial nutritional profile (Fernandez-Cassi et al. 2019). To mass rear insects for these purposes, it is vital to understand optimal densities for increasing individual size and survival (output) while reducing disease and stress. In this study, we investigated the impacts of various rearing densities on *A. domesticus* life-history features as well as their ability to survive prolonged starvation. We reared experimental groups for life-history analysis (growth rate and survival to maturation) at 0.09, 0.19, 0.47, and 0.93 cricket/cm^2. We determined that the number of individuals reaching maturation was significantly reduced in the 0.47 and 0.93 cricket/cm^2 density groups (Fig. 1). This represented a decline

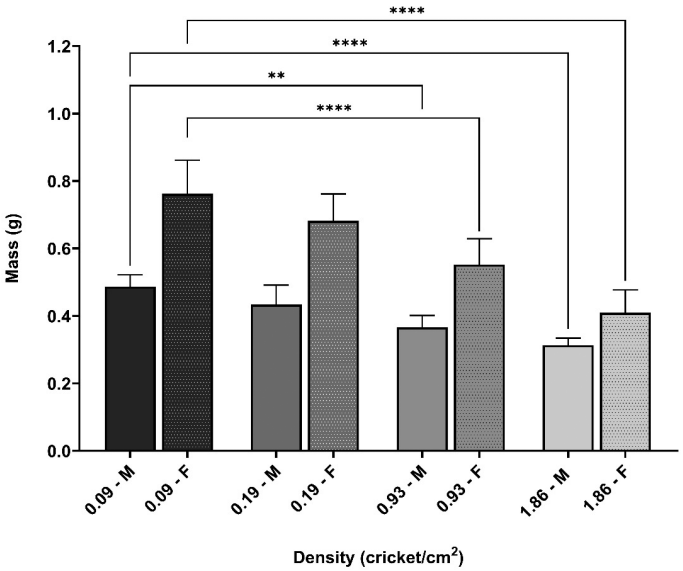

Fig. 3. Mass of adult *Acheta domesticus* used for starvation treatment from each density group. Values represent the mean mass of each group ± SD. The mass of each individual was recorded prior to starvation treatment. A one-way ANOVA indicated significant differences between groups ($F(7, 69) = 58.47$, $p < 0.0001$), with a Tukey's multiple comparison test indicating significantly reduced masses ($p < 0.0001$) in both the 0.93 and 1.87 cricket/cm^2 females compared to the 0.09 cricket/cm^2 female controls. Significant reductions in mass were also detected in the 0.93 ($p = 0.0018$) and 1.87 ($p < 0.0001$) cricket/cm^2 males compared to the 0.09 cricket/cm^2 male controls. Although not represented on the graph, between-sex differences ($p < 0.0001$) were also detected between males and females in the 0.09, 0.19, and 0.93 cricket/cm^2 groups.

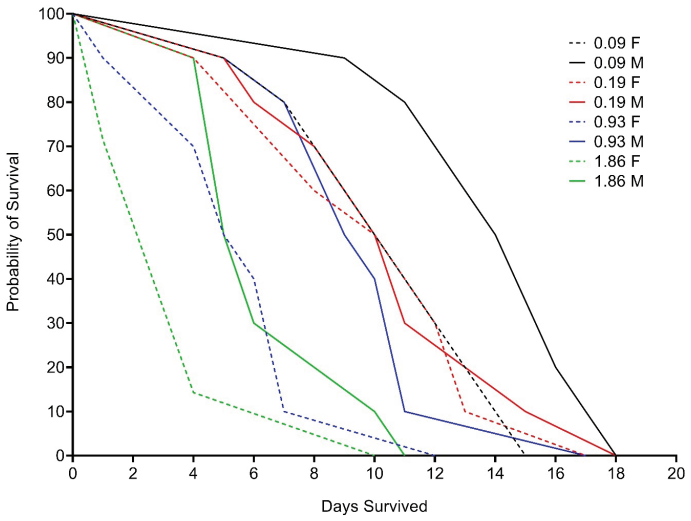

Fig. 4. Starvation resistance of adult *Acheta domesticus* reared at various densities. All density and control groups were separated at 14 days of age (4th instar) and maintained until adulthood. Three to four weeks post-maturation individuals were separated and deprived of sufficient food. The number of days survived was recorded for all crickets and represented as mean ± SD. Multiple regression analysis suggests both sex ($p = 0.0005$) and density ($p < 0.0001$) to be significant predictors of starvation resistance. The Gehan–Breslow–Wilcoxon test showed significant differences in survivorship between the 0.93 cricket/cm^2 ($p = 0.0030$) and 1.86 cricket/cm^2 ($p = 0.0008$) groups compared to the lowest density (0.09 cricket/cm^2) female group. Variation in survivorship was also evident in the 0.19 ($p = 0.0328$), 0.93 ($p = 0.0063$), and 1.86 ($p = 0.0001$) cricket/cm^2 groups compared to the lowest-density males.

of 31% and 45% compared to the lowest density 0.09 cricket/cm^2 group but still resulted in 142 and 224 individuals maturing, respectively. For growth rate, multiple regression analysis found that both sex ($p < 0.0001$) and density ($p < 0.0001$) have highly significant predictive power for growth rate. Interaction between sex and density ($p = 0.0345$) also had a slightly significant impact on growth rate (Fig. 2). A decline in growth rate was evident between the highest density males and females compared to their lowest density conspecifics, constituting a 5.18% and 12.45% decline, respectively.

Although our study did not indicate large declines in growth due to increased density, our results suggest that density is a strong predictor of growth rate. Prior studies have indicated that both increased mortality and decreased growth are expected due to overcrowding (Peters and Barbosa 1997, Zaelor and Kitthawee 2018). These impacts on survival and growth are likely due to increasing population density, which has been shown to increase competition, physical injury, and stress (Parry et al. 2017). In an early study on the rearing densities of larval American cockroach *Periplaneta Americana (*Wharton et al. 1967), increasing density reduced both survival and growth. Other studies show similar trends, although the magnitude of survival and growth reductions seem to vary among even closely related species. A study by Parry et al. (2017) found that both survival and growth were significantly affected by density; the relationship was not linear and was significantly different between the five blow fly species used. Studies on the English grain aphid *Sitobion avenae* (Xing et al. 2021) revealed decreases in both the growth and survival of early-instar nymphs with increased population density. The effects of density also seem to be age dependent, with some species being more resistant to density in later life stages. For example, adult density does not significantly affect survival or reproductive traits in *C. homnivorax* (Berkebile et al. 2006).

Varying results in the literature indicate that population density effects are highly complex and species-specific, with some species being more resistant to the negative effects of overcrowding than others (Xing et al. 2021). Synergistic effects, such as temperature and other environmental factors, should also be considered, as they may influence the effects of density, as was shown in a study using *Sitobion avenae* (Xing et al. 2021). This variability highlights the need for species-specific data on density impacts. It is also likely that a more substantial reduction in growth may have appeared at higher densities than used here. In addition, as shown in Fig. 1, it is likely that the use of even higher densities may not only further impact growth but also survival.

The second key aspect of this study was to investigate the impact of rearing density on starvation resistance. Intermittent food shortages are common in both the wild and in high-density mass rearing environments where competition for food is high (Zhang et al. 2019). Lack of food over extended periods of time can affect the growth, survival, and reproduction of individuals within the population (Zhang et al. 2019). Our chosen regression model suggested that sex ($p = 0.0005$) and to a larger extent density ($p < 0.0001$)—but not the interaction between the two—are significant predictors of starvation resistance (Fig. 4). Analysis of survivorship curves indicated that most groups showed significant differences in survivorship compared to their same-sex lowest-

density group. The survival rate of the males in the 1.86 cricket/cm2 density group was less than the males in the 0.09 cricket/cm2 control group. It is important to note, however, that the mass of individuals used in the starvation resistance experiments was significantly lower in the 1.83 cricket/cm^2 and 0.93 cricket/cm^2 groups for both males and females (Fig. 3). The 1.83 cricket/cm^2 density group was not measured for growth rate, but it is likely that at this extremely high density the growth rate would be reduced given the significant reduction in mass observed before starvation. Our results are in line with to the manner in which insects are able to resist starvation in conditions in which migration is not feasible. Under starvation conditions, insects will undergo physiological modifications that alter their metabolism to help them cope (Zhang et al. 2019). They will first metabolize blood sugar (trehalose) and then lipids (triglycerides) to improve hunger resistance (Zhang et al. 2019). It is therefore likely that the reduction in mass observed in the 1.83 cricket/cm^2 and 0.93 cricket/cm^2 disadvantaged these individuals in terms of their ability to break down sugar and fat stores as effectively as larger individuals.

Although not always considered, sex often plays a key role in stress-related impacts on life-history features. Our results for both growth and starvation resistance showed significant contributions of sex on both variables (Tables 2, 3). Increased density has been shown to not only affect life-history traits but also alter interactions between different sexes (Rull et al. 2012, Parry et al. 2017). This may lead to alterations in fecundity and fertility (Rull et al. 2012). Sex differences are expected, as females of this species are typically larger than males (Lyn et al. 2012). For starvation resistance, sex differences were evident in the 1.86 and 0.93 cricket/cm^2 groups, with females being more sensitive to starvation than males (Fig. 4). However, most studies have found female insects to be generally more resistant to food-related stress, as females are typically larger and therefore have more nutrient stores. For example, Gaskin et al. (2002) found male *Ceratitis capitata* to be more negatively impacted by increases in density than females. The researchers postulated that this was due to increased aggression and behavioral costs to mate successfully in males (Gaskin et al. 2002). A study conducted on five species of blow flies reared at various densities indicated that females survived longer than males across all species used (Parry et al. 2017). Higher mortality in males versus females due to density variation has been recorded in *Lucilia* (Parry et al. 2017) *sericata* and *Ceratitis capitata (*Gaskin et al. 2002), Males of *Cochliomyia homnivorax* (Berkebile et al. 2006, Pitti et al. 2011) tend to show increased mortality under several rearing conditions, including high density, protein rich diets, and high temperature . While it is unclear why females in our study were more sensitive to starvation, trade-offs between cell maintenance and repair, resulting in aging and death, and the energetic costs of egg production have been documented in female insects (De Loof 2011). The females used in this study were sexually mature adults, potentially making them less able to mitigate the impacts of starvation.

These results have profound implications for insect farming in which productivity is often deterred by the increased competition and stress associated with high density (Zaelor and Kitthawee 2018). Our results indicate that optimal densities for the mass rearing of *A. domesticus* are likely to be < 0.93 cricket/cm^2, as this minimizes reductions in growth and maintains adequate resistance to starvation in adulthood. In addition, although survival to maturation is significantly reduced at this density, the number of individuals that do survive, in this case 224, is significantly greater than at densities with < 250 individuals. Thus, as the goal of mass rearing is to produce the largest number of individuals at the maximum body size while reducing stress, we believe that < 0.93 cricket/cm^2 is optimal. These results should guide future mass-rearing endeavors to optimize production while reducing mortality and other negative effects of overcrowding. It is also recommended that future research focus on a diversity of endpoints, such as how density influences reproductive output, immune responses, and survivorship in this species to further inform optimal rearing densities. Synergistic effects between density and other environmental factors such as temperature, humidity, etc., should also be investigated.

Acknowledgments

This work was funded by the NSERC Discovery Grant (RGPIN-05693-2015).

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