Published in final edited form as:

Med Vet Entomol. 2011 December ; 25(4): 445–453. doi:10.1111/j.1365-2915.2011.00950.x.

TEMPERATURE INDUCES TRADE-OFFS BETWEEN DEVELOPMENT AND STARVATION RESISTANCE IN *Aedes Aegypti* (L.) LARVAE

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Abstract

While heightened temperature increases the development rate of mosquitoes, for *Aedes aegypti*, larvae that commonly experience food limitation in urban habitats, temperature effects on adult production may also be influenced by changes in the capacity of larvae to survive without food. We carried out experiments at 2°C intervals between 20 and 30°C on the growth, maturation rate and the longevity of optimally fed larvae placed in starvation. Overall, both growth rate and starvation resistance were lower in the first three larval instars (L1-L3) as compared to L4, in which greater than 75% of growth occurred. While increased temperature reduced the duration of each instar, it had a U-shaped impact the effect of initial growth on starvation resistance, which increased from L1 to L2 at 20 and 30°C, remained constant at 22 and 28°C, and decreased at 24 and 26°C. Growth from L2 to L3 significantly increased starvation resistance only from 26–30°C. Increased temperature (above 22°C) consistently reduced starvation resistance in L1. In L2-L4, 2°C increments decreased starvation resistance between 20 and 24°C, but had weaker and instar-specific effects above 24°C. These data show that starvation resistance in *Ae. aegypti* depends on both instar and temperature, generating a tradeoff between increased development rate and reduced starvation survival of early instar larvae, particularly in the lower and middle temperatures of the dengue endemic 20–30°C range. We suggest that anabolic and catabolic processes in larvae have distinct temperature dependencies, which may ultimately cause temperature to modify density regulation of *Ae. aegypti* populations.

Introduction

Water temperature may vary broadly in the larval container environments of *Aedes aegypti* (L.) and, thereby, may affect numerous characteristics that determine efficient transmission of arboviruses, including development rate, body size and survival. Laboratory experiments consistently show that in the 20–30°C range *Ae. aegypti* conforms to the classical temperature-size rule (TSR) in ectotherms in which development rate increases linearly, and adult size decreases monotonically, with increasing rearing temperature (Gilpin & McClelland, 1979; Rueda, Patel, Axtell, & Stinner, 1990; Tun-Lin, Burkot, & Kay, 2000). Field studies, however, have not revealed consistent patterns between *Ae. aegypti* production and temperature variation in the 20–30°C range. Increasing temperature in the 22–30°C range has been negatively associated with container mosquito production in Puerto Rico (Barrera, Amador, & Clark, 2006) and Australia (Tun-Lin et al., 2000) and positively associated in Brazil (Favier et al., 2006). Elucidating the determinants of these opposing findings requires understanding how temperature interacts with the ecological processes that

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drive variation in mosquito production in local habitats (Padmanabha, Soto, Mosquera, Lord, & Lounibos, 2010).

Food limitation in developing larvae plays an important role in shaping *Ae. aegypti* populations in urban areas where this species transmits dengue viruses. Its principal habitat, water-containing household vessels, is notoriously resource poor in comparison to common laboratory rearing conditions (Arrivillaga & Barrera, 2004; Barrera et al., 2006; Strickman & Kittayapong, 2003; Tun-Lin et al., 2000). Larval food indicators such as exposure to vegetation, rainwater and human food detritus have been positively associated with *Ae. aegypti* pupal abundance or the dynamics of production in field containers (Barrera et al., 2006; Hammond et al., 2007; Morrison et al., 2004; Subra & Mouchet, 1984). Moreover, larval mortality has been found to regulate the abundance of adult female *Ae. aegypti* (Southwood et al, 1972), which is not usually affected by predation of larvae or interference competition in urban vessels (Dye, 1982; Gilpin & McClelland, 1979). Thus, the capacity of larvae to persist to adult emergence in the context of food limitation may be an important determinant of human-vector contact rates in dengue endemic areas.

The physiological effects of food limitation on mosquito development are dependent upon larval energy reserves. By depleting energy reserves, starvation will directly induce mortality when larvae are no longer able to maintain basal metabolic functioning (Wigglesworth, 1942); however starvation does not reduce the overall body mass of larvae (Telang, Frame, & Brown, 2007). Energy depletion may occur through density dependent resource competition with other larvae (Gilpin & McClelland, 1979), and/or through the potential metabolic costs of searching for food in large volume vessels. Similarly, food limitation reduces development rate by prolonging the attainment of a critical level of energy reserves, which are likely to trigger the physiological processes resulting in pupation (Chambers & Klowden, 1990; Gilpin & McClelland, 1979; Telang, Frame, & Brown, 2007). A longer developmental period, in turn, may indirectly lead to increased larval mortality from stochastic external processes such as human behavior (Padmanabha et al., 2010). Thus, maintaining a favorable energy balance is critical to the ability of *Ae. aegypti* to survive and mature in food-limited aquatic habitats.

While higher temperature increases development rate (Rashed & Mulla, 1989), little is known about how mosquito larvae obtain the energy required to mature faster in warmer conditions (Lafferty, 2009). Higher temperature undoubtedly increases energetic demands in ectotherms by increasing the rate of biochemical reactions (Schoolfield, Sharpe, & Magnuson, 1981). If small increases in temperature were to cause a net change in the accumulation of energy stores in feeding larvae, it would affect survival in the absence of food. Moreover, a net energy deficit in higher temperatures may increase the time required for larval *Ae. aegypti* to obtain the resources necessary to mature in common urban habitats. This would suggest an interactive effect of temperature and food limitation on *Ae. aegypti* production that would be undetectable in temperature experiments that employ optimal feeding conditions (Gilpin & McClelland, 1979; Rueda et al., 1990). Understanding how temperature affects the capacity of larvae to withstand starvation is therefore central to predicting how climate variation influences *Ae. aegypti* production.

In all organisms metabolic processes depend on the stage of development; for mosquitoes this means that energy uptake and expenditure may vary across larval instars. Given paradoxical evidence that warmer temperature *increases* growth efficiency but *reduces* final size in most ectotherms and in *Ae. aegypti* in particular (Rashed & Mulla, 1989), the TSR is has been suggested to result the effects of temperature on the trajectory of growth and development (Angilletta & Dunham, 2003; Kozłowski, Czarnoleski, & Danko, 2004). As such, in developing mosquitoes temperature may have differential effects on energy

accumulation and depletion among the four larval instars. This may result in complex, instar-specific effects of temperature on starvation resistance in *Ae. aegypti*, particularly given evidence that at constant temperatures, survival without food varies non-linearly among mosquito instars (Rasnitsyn and Yasyukevich, 1989). The potential consequences of such instar-specific temperature effects for *Ae. aegypti* production in resource limited habitats are underlined by competition studies showing that early instar larvae are more sensitive to the density of later instars than vice versa (Gilpin & McClelland, 1979).

In order to explore the potential costs of warming temperature on *Ae. aegypti* development we carried out a series of experiments on growth, maturation rate and starvation resistance in larvae reared at 2°C increments in the dengue endemic 20-30°C range. In particular, we focused on the impact of temperature on the survival of optimally fed larvae transferred to foodless conditions, since this trait is the outcome of multiple metabolic processes that define the net accumulation of energy reserves during feeding and rate of energy depletion upon starvation. We asked (1) how does increasing larval instar affect starvation resistance in each temperature and (2) how do increments in temperature affect starvation resistance in each larval instar. These questions have direct implications for how rising temperature may modify the regulation of *Ae. aegypti* populations in heterogeneous urban environments.

Materials and methods

All experiments were conducted using an *Ae. aegypti* strain (F3) from Barranquilla, Colombia (altitude 5 m, latitude 10 °N, mean monthly temperatures 26-29°C), in incubators calibrated to 20, 22, 24, 26, 28 and 30°C. At each temperature, experiments were carried out in one incubator in which water temperature was measured hourly in a 5 ml cup, using an I-button temperature logger (Embedded Data Systems, Lawrenceburg, KY). Prior studies have shown that food composition influences starvation resistance in *Ae. aegypti* (Barrera, 1996). To prepare a standardized household detritus for food treatments, we distributed plastic bags in 30 homes located in a dengue endemic neighborhood in the Colombian city of Bucaramanga. Because the detritus content in domestic vessels is likely to depend on the “naturally” occurring household debris in part of the house containing the vessel, we asked residents to sweep the area immediately surrounding their water storage vessel and deposit in a plastic bag the contents obtained over a 24 hour period. Bags were obtained from 25 houses. After removing big rocks and synthetic substances, a 0.8 kg mixture resulted, composed (by weight) of 52% dirt/sediment/small rocks, 11% leaves and stems and 37% diverse organic matter (fibrous material/hairs, insects, human/pet food, seeds). We ground this mixture into “sediment” in order to visually minimize biases in food application across containers and over time. Contents were dried, and a manual grinder was used to convert > 95% of the mixture into particles capable of passing through netting with pores smaller than 0.25mm². The remaining larger particles were discarded. Upon sifting, the resulting mixture consisted of a high density granulose bottom layer and a fibrous top layer. Food applied to each feeding cup included both layers and was measured on a balance with approximately ±5mg error. No *a priori* information was available on the quality of this food, but based on the observed development rates (see below), it appeared comparable to laboratory studies of *Ae. aegypti* using ample quantities of liver powder (Rueda et al., 1990).

In all feeding regimes, 100 mg of detritus were pre-incubated for 24 h in 0.9 L of water (1L feeding cups) in order to allow more time for microbial development as the sediment had been freeze dried. Ten newly hatched (0-6 hrs old) first instar larvae (L1) were added to each feeding cup, and another 100 mg of food were added after 48 h. These conditions were chosen specifically to minimize resource competition and the time spent searching for food prior to starvation. As such our experiments focused on the capacity of optimally fed larvae of each instar to survive foodless conditions.

After feeding for a specified number of days, larvae were individually transferred to 4 ml of distilled water (in 5ml cups) and monitored daily until death or pupation. Transfers of larvae (n=40) from four replicate feeding vessels were carried out daily beginning with newly hatched larvae (that were never fed) on Day 0, until the day (specific to each temperature) in which pupae were observed in the feeding cups. For data analysis, we used the instar of each individual upon transfer to distilled water, although in some instances larvae molted after transfer to distilled water. However, experimental starvation of well fed larvae is known to artificially induce molting in *A. aegypti* (Nishiura, Burgos, Aya, Goryacheva, & Lo, 2007; Telang et al., 2007); given our focus on the trajectory of starvation survival capacity over the course of development under constant, optimal food conditions, we did not include molting of starved larvae in our analysis. Observations of pupation and mortality were made daily, and no larval mortality in the feeding cups was observed.

We analyzed separately the effects of each 2°C temperature increment on the risk of death in each of the four larval stages (instar upon transfer to distilled water), and the effects of each instar increment on starvation resistance in each of the six temperatures. We chose this pairwise approach because of the biological and ecological relevance of understanding how starvation survival capacity varies sequentially between developmental stages and 2°C changes in temperature that commonly occur seasonally in dengue endemic climate. Moreover, given visual inspection of the data indicating non-monotonic, non-linear and potentially interactive effects of temperature and larval instar on starvation resistance, this approach preserved the simplicity and interpretability of the analysis. Thus for each of the five temperature increments (20-22, 22-24, 24-26, 26-28, 28-30°C), we tested four different hypotheses on starvation resistance, one for each instar; similarly, for each of the three instar increments (L1-L2, L2-L3, L3-L4) we tested six different hypotheses, one for each temperature.

Differences in the pairwise mortality hazard were tested using maximum likelihood Weibull survival models, with STATA 8.0 (StataCorp, 2001, Statistical Software: Release 8.0, Stata Corporation, College Station, TX), in which the odds of death in a particular instar or temperature were compared to the next lower instar or temperature group. The Weibull model readily accommodates survival data in which the mortality hazard changes over time, assuming that the shape of the hazard function is constant across covariates. In these starvation experiments, mortality hazards increased over time in distilled water, based on the work of Wigglesworth (1943) demonstrating that over time starved larvae have increasingly fewer energy reserves to maintain basal metabolism. Shared, gamma-distributed frailty was assumed for the survival of larvae that fed in the same cup. Shared frailty models for clustered survival data are analogous to random effects models for continuous outcomes. A step-down Sidak correction (Ludbrook, 1998) was employed in order to correct for the increased Type I error associated with testing for multiple hypotheses. The Sidak correction sets the p-value at $1-(1-\alpha)^{1/k}$, where k is the number of independent hypotheses tested for each predictor. The step-down procedure increases power by excluding highly significant hypotheses, such that initially k is equal to the total number of hypotheses, but after the first null hypothesis is rejected, the Sidak significance level is re-calculated based on the remaining $k-1$ hypotheses, and so forth.

In addition to the starvation resistance experiment we verified that our experimental conditions reproduced observations of prior studies in *Ae. aegypti* regarding temperature effects on development rate, final size and weight trajectory (Rueda et al., 1990; Telang et al., 2007). Measures of development rate included average feeding time of each instar, feeding days of larvae that pupated after transfer to distilled water (hereby referred to as commitment to pupation) and time to pupation in feeding larvae. For this latter measurement, feeding was continued in four replicate feeding cups at each temperature until

the day in which 50% of the larvae had pupated. We did not record pupation percentage daily, only on the day in which we counted at least 20 pupae among the four feeding cups, at which point the experiment was discontinued. Although the sex of emergent adults was not quantified, pupae in both the starvation and feeding groups were likely to be predominantly males (see results and discussion). In a subsequent experiment with identical feeding and temperature conditions, we measured the dry weight of late L4s, using 3 replicate feeding cups (n=30), individually weighing larvae after 24 hours in a drying oven. Weighing occurred on the day prior to the previously recorded days to 50% pupation at each respective temperature. We chose this measurement time, rather than weight at pupation, in order use the data to fit a mechanistic model of *Ae. aegypti* development (*manuscript in preparation*). All larvae and pupae were weighed in each temperature group, so as to avoid the biases in sex ratios incurred in the measurement of time to pupation. Temperature effects on late L4 weight were evaluated using ANOVA, controlling for feeding cup. We also measured the dry weight of larvae killed daily after feeding from 0 to 4 days at 28°C. Because the error in our microgram balance was comparable to the weight of early instar larvae, 0-3 day fed larvae were weighed in ten different groups. Group size was 10 for unfed larvae and 5, 4 and 2 for 1, 2 and 3-day fed larvae, respectively; this corresponded to five, four and two replicate feeding cups for each respective feeding time. In 4-day fed larvae the weight measurement corresponded to the late L4 weight measurement at 28°C. Larval instars after each feeding day were the following at 28°C: one day: L2, two day: L3, three and four days: L4. There was 100% synchrony in the instar among the larvae reared for weighing daily at 28°C, with the exception of 1 pupa that was included in the weighing of 4-day fed larvae.

Results

Development rate increased with higher temperature using a variety of measures. In feeding larvae, time to reach at least 50% pupation at each temperature (observed pupation percent) was 9 days at 20°C (52.5%), 8 days at 22°C (50%), 7 days at 24°C (82.5%), 6 days at 26°C (100%), 5 days at 28°C (60%), 5 days at 30°C (92.5%). Feeding time required to pupate after transfer to distilled water also decreased monotonically with increased temperature (Figure 1), with over 60% of larvae committed to pupation after 3 days feeding at 30°C, whereas at 20°C feeding larvae needed 7 days to reach this level of pupation (Figure 1). The average number of feeding days for each of the four larval instars increased with lower temperature (Figure 2). For example at 20 and 22°C both zero and 1-day fed larvae were L1, as compared to only unfed larvae in the higher temperatures, resulting in a larger sample of starved larvae (Figure 2). In L3 and L4 at 20°C, mean feeding time was greater than double that of 30°C. The higher rate of commitment to pupation at 30°C (Figure 1) resulted in fewer L3 (n=25) and L4 (n=29) that experienced starvation mortality (Figure 2).

Mean mass in late L4 decreased with increased temperature ($F_{1,5}=16.7$, $p<0.001$). The decrease was steeper between 28 and 30°C, although mean weights decreased monotonically with increased temperature (Figure 3). Dry weight through the course of larval development at 28°C showed a roughly sigmoidal trajectory, with exponential growth occurring between 2 and 3 days feeding and considerably slowing of weight gain between 3 and 4 days feeding (Figure 4). Roughly 75% of final weight was gained in L4.

Overall, we observed that the effects of 2°C increases in temperature on starvation resistance varied by larval instar and across the 20-30°C range. In L1 mean survival was highest at 22°C and decreased as temperature rose to 30°C, but was markedly lower at 20°C (Figure 5a). In L2 and L3, survival was noticeably higher at 20 and 22°C, but comparatively varied much less with temperature between 24 and 30°C. In L4, there was a non-monotonic relationship between temperature and survival with highest survival at 20°C and notably lower survival at both 24 and 30°C. (Figure 5a). Grouped by temperatures, Figure 5b shows

that starvation survival capacity increased from L1 to L2 at 20, 22, 28 and 30°C, but decreased at 24 and 26°C. At all temperatures, survival starvation during increased between L2 and L4, with a particularly large increase from L3 to L4 (Figure 5b).

Pairwise comparisons of mortality between sequential instars, represented by the hazard ratio of the higher versus lower instar (>1 means increased mortality in the higher instar, <1 increased mortality in the lower instar) showed a bell-shaped relationship between increasing temperature and the effects of early development. Mortality rate of L2 as compared to L1 was significantly lower in the extreme temperatures (20 and 30°C), similar at 22 and 28°C and significantly higher in the middle temperatures (24 and 26°C) (Figure 6). L3-L2 hazard ratios were not significantly different from 1 between 20-24°C; from 26-30°C they were significantly lower than 1, indicating reduced mortality in L3 as compared to L2 in the warmer temperatures (Figure 6). All temperatures exhibited significantly lower mortality of L4 as compared to L3, with hazard ratios well below 1 (Figure 6).

Notwithstanding significant stage-specific effects, Figure 7 shows that in general increased temperature between 20 and 24°C tended to increase mortality rate, whereas 2°C increments between 24 and 28°C had lesser effects and more frequently reduce mortality. Mortality once again increased from 28 to 30°C. However each 2°C increment had unique instar-specific patterns. For both the 20-22°C and 24-26°C comparisons mortality increased with warmer temperature in L1, but decreased in L4 (Figure 7). Mortality sharply increased from 22 to 24°C in all instars with larger effects in L2, L3 and L4. Starvation resistance was similar between 28 and 26°C except in L2, where mortality at 28°C was lower than at 26°C. By contrast both L2 and L4 had sharply increased mortality rates at 30 as compared to 28°C. The Sidak correction removed only one model that would have otherwise been significant at $\alpha=0.05$, had we not corrected for increased Type 1 error (28 vs. 26°C in L1, Figure 7). All significant declines in death hazards in higher temperatures or instars had upper 95% confidence limits of hazard ratios ≤ 0.75 , and significant increases had lower 95% limits ≥ 1.19 .

Discussion

Although temperature is a key ecological determinant of variation in the development rate and bodysize of *Ae. aegypti* populations, surprisingly little is known about the potential developmental costs that increased temperature may have for this important disease vector. Understanding how the survival of experimentally starved larvae varies over the course of development is useful in exploring potential tradeoffs of increased temperature for two reasons: (1) given prior studies relating starvation resistance and energy reserves (Wigglesworth, 1942; Telang et al, 2007), our experimental system of feeding and starvation is likely to reflect the balance between the net energy reserves accumulated during feeding and the rate of energy depletion required to maintain basal metabolic functions during starvation, and (2) starvation resistance over the course of development bares direct implications for the capacity of *Ae. aegypti* to mature to adult in resource limited conditions. We found that while increasing temperature between 20 and 30°C consistently and monotonically increases the development rate of each larval instar, it has more detrimental effects on starvation resistance in the 20-24 as compared to 24-30°C. In particular, we observed that temperature significantly influenced the trajectory of starvation resistance in early instars, such that growth and development in optimal feeding conditions had reduced and delayed effects on improving starvation resistance in the middle temperatures of the 20-30°C range. These data suggest that distinct temperature dependencies of the various metabolic processes regulating *Ae. aegypti* development cause larger tradeoffs between development rate and starvation resistance in early instars in the middle temperatures of the 20-30°C range. Due to the importance of density-dependent mortality of early stage larvae

in regulating *Ae. aegypti* populations (Southwood, et al, 1972), our results suggest that temperature variation on the order 2°C can potentially have profound effects on *Ae. aegypti* population dynamics.

Because the longevity of starved larvae depends on the exhaustion of energy stores (Wigglesworth, 1942), survival of larvae after transfer to distilled water is largely determined by basal metabolic rate, the net accumulation of energy reserves during feeding and the weight prior to starvation (Angilletta & Dunham, 2003; Gilpin & McClelland, 1979). Temperature effects on L1, which were never exposed to a food source (except for 50% of larvae starved at 20 and 22°C that fed for 24 hours, Figure 2), are likely to correlate well with the thermal dependence of basal metabolism. This is because it is unlikely that the weight of newly hatched larvae correlates with temperature, given the resistance of *Ae. aegypti* eggs to environmental conditions and that eggs (F3) were derived from a common source population and stored at a common temperature. We observed decreasing starvation resistance of L1 as temperature increased from 22-30°C, but reduced starvation resistance at 20°C. This latter result might be due to limited cold tolerance of the tropical, sea level *Ae. aegypti* strain used, and as such, may not be observed in mosquitoes adapted to cold weather. Accordingly, these data are consistent with general metabolic theory in ectotherms, based on enzyme kinetics, that heat increases basal metabolic expenditure (Sharpe & DeMichael, 1979).

As larvae grow and develop, the capacity to resist starvation is likely to be less sensitive to basal metabolic rate, and more sensitive to the efficiency of energy storage and how metabolic processes scale with increasing weight. Metabolic rate is well known to scale less than linearly with body size in all organisms and has been suggested to be proportional to a 2/3 exponent of total body mass in *Ae. aegypti*, although it may also be sensitive to temperature (Gilpin and McClelland, 1979; Angilletta & Dunham, 2003; Kozłowski et al., 2004). Given the sigmoidal growth trajectory of *Ae. aegypti*, in which 75-80% of final mass is attained in the final larval instar (Figure 3; Telang et al, 2007), a metabolic exponent of 2/3 means that L4 growth is likely to be more energetically efficient than in earlier instars, due to proportionally less metabolic expenditure. This would explain why in all six temperature treatments, the largest increase in starvation resistance occurred between L3 and L4. Moreover, initial larval weight gain after hatching is likely to be the least energetically efficient, and therefore likely to engender greater costs in terms of starvation resistance. Accordingly, our finding of a U-shaped relationship between temperature and the effect of initial development (L1 to L2) on starvation resistance suggests that at 20°C initial weight gain produced a net increase in energy reserves due to reduced depletion (catabolic) rate, whereas at 30°C initial growth was also favorable, but due to increased energy storage (anabolism). As such growth from L1 to L2 reduced starvation mortality rate at these At 22 and 28°C there was a relative balance between energy storage and depletion, whereas at 24 and 26°C initial feeding and development led to larger increases in catabolism than in anabolism. Moreover, development from L2 to L3 had weak and insignificant effects on improving starvation resistance at 20-24°C, but caused strong improvements at 26-30°C, suggest that feeding L2 more readily store reserves at the higher temperatures. These hypotheses are further supported by our observations of reduced survival in newly hatched, never-fed larvae at heightened temperatures (suggesting a positive correlation between temperature and metabolic expenditure), as well as evidence that heightened temperature increases food uptake and conversion into biomass in *Ae. aegypti* (Rashes and Mulla, 1989). These data are consistent with a metabolic scenario in which temperature-induced increases in catabolic rate occur throughout the 20-30°C range, whereas increased initial anabolic rate occurs only above 26°C.

Survival without food is likely to be a key trait in *Ae. aegypti* larvae that affects pupation success and maturation rate in food-limited container habitats (Barrera & Medialdea, 1996). Understanding the effects of temperature on starvation resistance in early instars (L1-L3) vis-à-vis L4 has particular ecological implications because these experience the most density dependent mortality of any *Ae. aegypti* life stage (Southwood et al, 1972), and they are disproportionately susceptible to resource competition from later instars (asymmetric competition) (Gilpin and McClelland, 1979).. Here the observed starvation resistances support prior findings that L4 have larger competitive effects on L1 than vice versa in *Ae. aegypti* (Gilpin & McClelland, 1979), as L4 demonstrated a much larger capacity to survive foodless conditions than early instars at all six temperatures (Figure 5b). Moreover, the results suggest that within the 20-30°C range small increments in temperature on the order of 2°C may have variable impacts on the intensity of asymmetric effects. For example, starvation resistance in L2, L3 and L4 was significantly lower at 24°C as compared to 22°C, suggesting that this temperature increment may reduce development rate in food-limited vessels, but not necessarily impact inter-age competition. By contrast, larvae reared at 26°C as compared to 24°C had significantly increased mortality in L1, reduced mortality in L4 and approximately similar survival at L2 and L3, suggesting that a 24 to 26°C increment would respectively increase and decrease L1 and L4 mortality in food limited vessels. This could favor production in container habitats with non-overlapping larval age-cohorts that receive infrequent egg-hatching stimulus, but would increase competitive asymmetry in vessels with continuous egg-hatching. At 28°C, L2 survival markedly increased with respect to 26°C, whereas survival in the other instars remained similar. From 28 to 30°C, the decline in starvation resistance was much stronger in L4 than in the early instars. This suggests the potential for a reduction in competitive asymmetry from 26 to 30°C, as early instar larvae would have a larger capacity to persist in vessels until late instars pupate and resource availability increases. Similarly, at 22°C we observed reduced survival in L4 and increased survival in L1 in comparison to 20°C. Although limited cold tolerance of the *Ae. aegypti* strain used may have contributed to increased L1 mortality at 20°C, the large increase in L4 starvation mortality at 22°C could in itself alleviate the resource competition experienced by early instars. Such temperature-induced reductions in competitive asymmetry between larval instars may favor adult *Ae. aegypti* abundance by reducing density-dependent mortality (Southwood et al, 1972). These results, underline the potential for small temperature increments to dramatically alter the dynamics of density regulation of *Ae. aegypti* populations within and among urban containers.

A number of design issues may affect the generality of our results. For example, unlike a prior study on starvation resistance in *Ae. aegypti* carried out at 27°C (Rasnitsyn and Yasyukevich, 1989) we did not regularly transfer larvae into new distilled water. Thus, larval waste products may have promoted microorganism development in the 5ml starvation cups. We observed lower L1 and higher L4 survivals in both the 26 and 28°C treatments than Rasnitsyn and Yasyukevich (1989), although these authors used a smaller sample size and an older laboratory colony of *Ae. aegypti*. Moreover, if microorganism growth provided sustenance for larvae transferred to distilled water, we might expect increased starvation resistance in the higher temperature treatments, a phenomenon that was not observed. Another source of uncertainty was the lack of *a priori* information of the quality of the field collected household detritus employed. We chose this food because heterogeneous particles and microorganisms that larvae encounter in field habitats bear little resemblance to standardized laboratory diets, and it has been shown that dietary composition affects resistance to starvation in *Ae. aegypti* (Barrera, 1996). However, the median time to pupation in feeding larvae was similar to that recorded by Rueda et al (1990) at 20 and 30°C, in which the authors employed a high protein homogeneous larval food. Finally sexual dimorphism in development rate and pupation may have given rise to sex ratio biases in some of the starvation groups. While sexual dimorphism in pupation timing, critical and

final mass is well described for this species (Chambers & Klowden, 1990; Timmermann & Briegel, 1999) little is known about how sex affects larval survival or the trajectory of development in early instars. Because males pupate earlier than females, most of the pupae represented in Figure 1 were likely male. However, a corresponding female bias in starved L4 that did not pupate is not necessarily true since the L4 that fed for fewer days may have been largely male. For example, 16 of the 29 starved L4 larvae that did not pupate at 30°C (and were therefore the subjects of the starvation resistance analysis) had fed for only two days. Therefore, while the 3 day-fed L4 that experienced starvation mortality at 30°C were likely to be females (13 of 29), males were likely to comprise the 2-day fed L4. In the absence of direct evidence, given that L4 males accumulate lipids more efficiently and weigh less than L4 females (thereby requiring fewer reserves to maintain basal metabolism) (Bedhomme, Agnew, Sidobre, & Michalakis, 2003; Chambers & Klowden, 1990; Timmermann & Briegel, 1999), we suspect that they may have heightened starvation resistance. This means that if groups of starved L4 that did not pupate indeed had female-biased sex ratios (an assertion that is difficult to support), the actual disparity (i.e. if sex ratios were 50:50) in L4 starvation resistance as compared to other instars would be even greater than that observed.

In this study we show that increasing temperature in the 20-30°C range monotonically increases development rate in all instars of *Ae. aegypti* larvae, but has non-monotonic and instar-specific effects on starvation resistance. The data strongly suggest that temperature has distinct effects on growth rate, anabolism and catabolism of stored energy in this important disease vector. These results call into question two fundamental assumptions of current models predicting that warming temperature will uniformly increase *Ae. aegypti* development rates (Focks, Haile, Daniels, & Mount, 1993; Jetten & Focks, 1997): (1) that temperature affects on development rate and mortality act through a single rate controlling enzyme whose activity increases monotonically with temperature in the 18-32°C range (Schoolfield et al., 1981) and (2) the dynamics of intra-specific resource competition are temperature independent (Gilpin and McClelland 1979). Our data represent a first step in developing a more complete understanding of how temperature interacts with density dependent regulation in *Ae. aegypti*. We intend to build upon this study by using the data to parameterize mechanistic models of *Ae. aegypti* growth and development at different temperatures. Ultimately, these models will allow us to study how a warming climate modifies ecological processes that drive the production of *Ae. aegypti* in dengue endemic areas.

Acknowledgments

We thank Roberto Barrera for reviewing the manuscript and Naoya Nishimura and Richard Escher for support in conducting the experiments. This study was partly funded in part by the GEF/World Bank Integrated National Adaptation Pilot (INAP), at the Instituto Nacional de Salud de Colombia and by NIH grants R01 AI- 042164 to CCL and R01 AI 044793 to LPL.

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Figure 1.

Commitment to pupation of larvae starved after indicated feeding times. Larval mortality during starvation accounts for trajectories with less than 100% pupation. Curves at each temperature terminate on the day in which pupae first appear before transfer to starving conditions.

Figure 2.

Average number of days feeding observed for each instar. Numbers above error bars (95% CI) are the total number of larvae transferred into distilled water, including both larvae that pupated (Figure 1) and starved (Figure 5). 40 larvae were transferred daily beginning with newly hatched larvae (feeding day 0) until the day in which pupae were observed at each temperature. Temperature(°C)–feeding days completed: 20-6, 22-5, 24-4, 26-4, 28-4, 30-3)

Figure 3. Mean W_{L4} ($\pm$ 95% CI) among temperatures. Measured after 4, 5, 6, 7 and 8 days feeding at 30, 28, 26, 24, 22, 20°C respectively.

Figure 4.

Weight trajectory at 28°C. Error bars show standard error of dry weight across groups of larvae multiplied by group size (n) in each feeding group as follows: newly hatched L1, (n=10), 1-day fed L2 (n=5) and 2-day fed L3 (n=4) and 3-day fed L4 (n=2); L4 (and 1 pupa) in the 4-day fed group were weighed individually (n=30).

Figure 5. Mean starvation survival (days $\pm$ 95% CI) among larval instars and temperature treatments (a) grouped by instars, (b) grouped by temperatures

Figure 6.

Effects of increasing larval instar on starvation resistance at each temperature, as indicated by hazard ratios. Greater than 1 on the y-axis indicate increased hazard of death at higher as compared to lower instar, below 1 indicates a reduced hazard. Error bars (95% CIs) that do not cross $y=1$ indicate significance at $\alpha=0.05$; “*” indicates significance using a stepwise Sidak correction to reduce type 1 error.

Figure 7.

Effects of 2°C increases in temperature on starvation resistance in each larval instar, as indicated by hazard ratios. Points above the $y=1$ axis indicate increased hazard of death at higher as compared to lower temperature, below 1 indicates a reduced hazard. Error bars (95% CIs) that do not cross one indicate significance at $\alpha=0.05$; “*” indicates significance using a stepwise Sidak correction to reduce type 1 error.