

INTERACTIONS OF TEMPERATURE WITH THE DYNAMICS OF *Aedes aegypti* (L.)  
DEVELOPMENT IN HOUSEHOLD VESSELS

By

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I dedicate this work to Richard Levins for stimulating my interest in understanding complexity.

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The mosquito *Aedes aegypti* (L.) transmits dengue and chikungunya viruses among human hosts in cities at temperatures ranging 20-30°C, where it thrives in water that humans store in vessels. Two processes are known to drive the dynamics of *A. aegypti* production in these habitats: resource limitation and human behavior. Although temperature affects mosquito size and maturation rate, there is little understanding of how temperature may affect the capacity of mosquitoes to develop in food limited, domestic environments. In this dissertation we use modeling, experimental and field approaches to explore the general hypothesis that the effects of human and resource-mediated processes on the size, development rate and pupation success of *A. aegypti* may be modified across temperature gradients or cities that vary in temperature. We experimentally show that due to increased energy demands, larvae at higher temperature may experience tradeoffs between development rate, size and starvation resistance. Moreover, our model of growth and energy storage in mosquitoes indicates that these tradeoffs can both explain the commonly observed effects of temperature on mosquito size and result in interactive effects of food and temperature on development rate. A food-temperature developmental interaction was morphologically corroborated by analyzing wing and epidermal cell size in experimentally

reared *A. aegypti*. Our data suggest that in resource poor habitats commonly inhabited by urban *A. aegypti*, development rate may be lower at 24-26°C in comparison to 20-22 or 28-30°C, thereby challenging the fundamental assumptions of widely used temperature-driven models of *A. aegypti* dynamics. Our field study in dengue endemic areas of Colombia indicates that the outcome of this food-temperature interaction depends on specific household behaviors, including the frequency of using, emptying and placing lids on domestic water. Moreover, each of these acts in the context of particular socio-cultural perceptions, altitude, urban architecture and water supply. Nonetheless, our data suggest that climate-induced decreases in water security predicted throughout Colombia may indirectly increase *A. aegypti* abundance through changes in human behavior. These results demonstrate the underlying interactions between human and mosquito responses to a changing environment and enhancing our capacity to predict *A. aegypti* production and design locally adapted intervention strategies.

## CHAPTER 1 INTRODUCTION

The mosquito *Aedes aegypti* (L.) is a major global vector of the two most prevalent arboviral diseases in the world, dengue fever (DF) and chikungunya fever, and continues to play a role in the transmission of urban yellow fever. This mosquito is uniquely adapted to environments where humans reside, both in aquatic stages that inhabit anthropogenic vessels and as adults that feed primarily on human blood. Human transportation networks and massive urbanization following the failed eradication campaign in the 1950-60s have contributed to burgeoning *A. aegypti* populations and persistent DF infection in most neotropical cities (Gubler, 2002). Colombia, for example, reports an average of over 40,000 DF cases each year (National Institute of Health of Colombia, 2008), hyperendemic circulation of all four DF serotypes, and currently has the highest dengue hemorrhagic fever (DHF) incidence rate in South America (Pan American Health Organization, 2008). Given the costs and ineffectiveness of insecticide spraying, reducing the rate of adult production in the container habitats of *A. aegypti* immatures remains the best prevention strategy for mitigating DF, for which no vaccines or treatments are available (Farrar et al., 2007).

In Colombia close to 80% of the population lives in plateaus and foothills of the Andes mountains, where endemic DF circulates in diverse climates with mean temperatures ranging from 21 to 30°C. As in many of the world's neighborhoods where DF is endemic, domestic water storage vessels are the primary aquatic habitat of *A. aegypti* in Colombia (Padmanabha et al., unpublished data; Romero-Vivas et al., 2006). Most field studies of this habitat associate two processes with spatio-temporal variation in *A. aegypti* production and adult size: larval food limitation (Strickman and Kittayapong, 2003; Arrivillaga and Barrera, 2004; Morrison et al., 2004; Barrera et al., 2006b), and human water storage behaviors such as water use (Hammond et

al., 2007), vessel emptying (Subra, 1983), cleaning (Chan et al., 1998) and covering with lids (Morrison et al., 2004). These processes may act independently or interactively by inducing starvation, extending larval development time and/or eliminating aquatic stages before they emerge as adults. Although temperature is also a well documented determinant of development rate and size of mosquitoes, there is surprisingly little understanding of how it may interact with the processes that regulate *A. aegypti* production in dengue endemic environments. Knowledge of how the effects of food limitation and human behavior are mediated by ambient temperature can lead to more effective climate- and habitat-specific intervention strategies to reduce *A. aegypti* production. Moreover, there is a need to develop understanding of how mosquitoes will respond to a warming mean temperatures at different disease endemic altitudes.

This study uses a combination of field surveys, experimental manipulations, morphological analyses and simulation modeling to investigate how water storage practices and larval feeding affect larval development in *A. aegypti*. In particular, we explore how the effects of each is modified across a temperature gradient, focusing on the range of 20-30°C in which dengue is endemic in Colombia.

### **1.1 General Eco-Physiological Aspects of Development and Growth in *A. aegypti***

Insect development is regulated by the endocrine system as it responds to nutrient uptake and environmental conditions. The release of the hormone ecdysone triggers molting between immature stages and juvenile hormone (JH) titer controls the type of molt e.g., from larva to pupa or pupa to adult (Nation, 2008). Feeding and starvation experiments with fourth instar (L4) *A. aegypti* indicate that cessation of JH secretion occurs after a minimal L4 feeding time and attainment of a critical mass, which is higher in females than in males (Chambers and Klowden, 1990; Timmermann and Briegel, 1999; Telang et al., 2007 ). If larvae are starved such that they

do not attain this mass, JH levels do not fall sufficiently to induce metamorphosis (Nishiura et al., 2007). In *Drosophila melanogaster*, the critical weight does not correlate with food availability, although this has not been directly studied in *A. aegypti*. Increasing temperature increases *A. aegypti* development rate (Rueda et al., 1990) and decreases critical mass and final body size (Chambers and Klowden, 1990).

A large body of evidence suggests that the level of stored energy is a key factor regulating *A. aegypti* development. Wigglesworth (1942) observed microscopically that starved L4 *A. aegypti* died when lipid droplets disappeared from the body (from Gilpin and McClelland, 1979). Depletion of lipids, but not overall mass, in starved *A. aegypti* has since been repeatedly confirmed (Gilpin and McClelland, 1979; Timmerman and Briegel, 1999; Telang et al., 2007). In general studies employing biochemical assays of developing larvae have implicated energy reserves in diverse forms, including lipids and glycogen stores, in the mechanism that triggers pupation. (Gilpin and McClelland, 1979; Chambers and Klowden, 1990; Telang et al., 2007)

Lipids also appear to play a role in defining the characteristic sigmoidal weight trajectory in larval *A. aegypti*, with roughly 80% of growth occurring in L4 (Telang et al., 2007; Nishiura et al., 2007). When reared upon high protein laboratory diets, lipid content as a percentage of body mass has been shown to remain relatively steady or slightly decrease as the L4 stage progresses (Timmerman and Briegel, 1999; Nishiura et al., 2007; Telang et al., 2007). Moreover the lipid incorporation rate has been shown to decrease during the L4 stage, particularly during the interval between attainment of critical mass and maximum larval size, when almost 50% of final size is attained (Timmerman and Briegel, 1999; Nishiura et al., 2007). This is supported by findings of Dye (1982), showing that experimental data on the time to maturation was best reproduced by a mathematical model when a term representing decreasing food assimilation

efficiency with larval age was incorporated. Together these findings suggest that energy storage may directly or indirectly regulate survival, pupation and growth in *A. aegypti* larvae.

## **1.2 Water Storage Behavior and *A. aegypti* Larval Development**

Water storage vessels are unquestionably an important larval habitat of domestic *A. aegypti* and are often the most abundant and productive urban containers in a particular area (e.g., Southwood et al., 1972, Reuben et al., 1978; Barrera et al., 1993; Vu et al., 2005; Bisset et al., 2006; Romero-Vivas et al., 2006; Burkot et al., 2007; Hammond et al., 2007; Koenraadt et al., 2008).

Larvae in water storage containers experience a number of unique processes related to human water storage practices that do not occur in other container habitats of mosquitoes, such as discarded tires, that passively receive rainfall. The first is that they are purposely filled and drained by humans and experience a much larger fluctuations in water level at daily or weekly timescales (Koenraadt et al., 2008). This provides a much larger hatching stimulus for eggs, increasing the frequency of larval infestation across vessels and potentially reducing intraspecific resource competition by decreasing the size of larval cohorts that hatch synchronously.

The second is that the processes of water extraction, emptying and refilling generate a highly unstable and often nutrient poor environment for larval populations (Subra and Mouchet, 1984; Arrivillaga and Barrera, 2004) that is frequently subject to stochastic collapses when containers are emptied (Subra, 1983). Frequent emptying may eliminate aquatic stages before they emerge as adults, thereby reducing the rate of *A. aegypti* production (Subra, 1983). Other water storage behaviors, such as water use (Hammond et al., 2007), cleaning (Chan et al., 1998) and covering with lids (Morrison et al., 2004) may reduce mosquito production by limiting nutrient input, oviposition or egg retention on container walls. However, not all of these are

amenable for mosquito control in particular water storage contexts. For example, frequent water usage may undermine the effects of lid placement and result in higher mosquito infestation rates because lid placement may be less consistent in vessels where water is frequently extracted (Phuanukoonnon et al., 2005; Hammond et al., 2007). Residents may be unwilling to empty vessels if water is stored primarily to compensate for unpredictable interruptions in piped water (Barrera et al., 1993).

These water storage dynamics may contribute to the recurrent finding that in residential, urban areas *A. aegypti* production is highly variable at small spatio-temporal scales. Spatially, virtually all studies of pupae find that the majority of pupae are generated by only a few sampling units (days, containers or houses) whereas the majority of sampling units are not productive (Subra, 1983; Focks et al., 1995; Bisset et al., 2006; Koenraadt et al., 2008). Getis et al. (2003) found that, while pupae cluster in containers within specific houses, larval infested vessels in Peru was randomly distributed across houses, suggesting that the determinants of dengue vector production reside *within* the household level. Similarly, Subra (1983) corroborated that in a Kenyan village, household behaviors were responsible for the large daily variation in pupal production. While container maintenance may cause the majority of water storage vessels in a particular community to be devoid of organic matter and/or *A. aegypti* immatures, variation in household-container interactions may result in a few vessels that produce large numbers of pupae (Subra, 1983).

Clearly a deeper understanding of human-container interactions is essential for determining sustainable community-based interventions to reduce mosquitoes. Assessment of relationships between human behavior and vector production through the widely used cross-sectional pupal/demographic surveys (Focks and Chadee, 1997) is problematic, however. When

residents of communities with a history of dengue transmission and *A. aegypti* control programs are visited by vector control personnel, their self-perceptions, desire to please the interviewer, and the difficulties in quantifying sometimes unconscious interactions with containers all limit the accuracy of self-reported behaviors in surveys. (Scrimshaw, 1990; Kasprzyk, 2005) Moreover, surveys with predetermined variables may not reveal the subjective experience of individuals in a particular social and physical context that influences their behavior (Golafshani, 2003). In order to understand the ecological coupling of the dynamics of *A. aegypti* production and human behavior, longitudinal studies of households with direct, repeated observation of human behavior, such as those carried out in a Kenyan village in the 1970s (Subra, 1983; Subra and Mouchet, 1984), need to be carried out in modern, dengue endemic urban areas.

### **1.3 Food Limitation and *A. aegypti* Development**

There is ample evidence indicating that *A. aegypti* is frequently limited by the resources available in larval habitats. This has been observed in field populations of *A. aegypti* by comparing body size/mass indicators of field-collected pupae with those of laboratory populations reared across food gradients (Tun-Lin et al., 2000; Strickman and Kittayapong, 2003; Barrera et al., 2006a). Domestic *A. aegypti* larvae experience starvation in nature (Arrivillaga and Barrera, 2004) and have been shown to differ from its subspecies, the sylvatic *A. aegypti formosus*, in the increased ability of larvae to withstand starvation (Gilpin and McClelland, 1979). Other studies have correlated larval food proxy variables such as exposure to leaf drip, rainwater and human food particles to *A. aegypti* pupal production in field containers (Subra and Mouchet, 1984; Morrison et al., 2004; Barrera et al., 2006b; Hammond et al., 2007), indicating that larval food resources may limit adult population size.

While food limitation is common in natural *A. aegypti* habitats, the extent to which it is driven by the depletion of resource by competing larvae is unclear. Broadly speaking, food

limitation arises whenever larvae incorporate into biomass less food than their physiological capacity to process ingested food. Food limitation may arise without resource competition in various situations. If water volume is high and resources are present but diluted or aggregated to the extent that larvae spend most of their time and energy searching for food, then food limitation may be density-independent (Legros et al., 2009). For example, Subra and Mouchet (1984) found that addition of larval food dramatically increased pupal production in natural habitats, whereas addition of larvae did not affect production. Rashed and Mulla (1989) demonstrated that *A. aegypti* larvae ingest inert particles more than other mosquito species. This suggests that in habitats with abundant inert particles, *A. aegypti* larvae may saturate their guts to the point where food consumption is driven by the rate of digestion and excretion. Therefore if larvae do not readily distinguish between nutritive and non-nutritive particles and the matter ingested is largely inert indigestible particles, food limitation, as measured by starvation or body size, will arise independently of larval density

Few field studies have directly demonstrated density-dependent resource competition. In containers found in an enclosed Buddhist monastery in Thailand, Southwood et al. (1972) enumerated all life stages of *A. aegypti* monthly over a 1-year period. The authors used life table methods to infer that density-dependent mortality from egg to the second instar regulated the population dynamics. Barrera et al. (2006a) found that larval density was positively correlated with pupal output, but negatively correlated with size, indicating that competition was strong enough to reduce larval growth in the interval between attaining critical and maximum mass, but not strong enough to induce mortality. Where it does exist in the urban Neotropics, competition affecting *A. aegypti* is usually intraspecific, notwithstanding occasional co-occurrence of this

species with *Culex quinquefasciatus* in large containers with high organic content, such as storm drains and sewers (Legros et al, 2009).

Using the results of Southwood et al. (1972), Gilpin and McClelland (1979) constructed a detailed model of *A. aegypti* production that successfully reproduced effects of variation in food-larval ratio on pupation success in laboratory experiments. They modeled *A. aegypti* growth using an exponential function in which the fraction of larvae that cross the critical mass threshold increased with the food:larvae ratio until the system become saturated with food at a maximum pupation rate. In further experiments on interference and resource competition within and between larval stages, Dye (1982, 1984) largely corroborated the results of Gilpin and McClelland. The competition-driven formulation of larval growth used by Gilpin and McClelland served as the basis for subsequent models integrating temperature effects on *A. aegypti* production (Focks et al., 1993; Jetten and Focks, 1997; Williams et al., 2008; Kearney et al., 2009). This formulation, however, assumes that effects of food limitation are temperature independent and therefore cannot be applied to study food-temperature interactions.

#### **1.4 Temperature Effects on *A. aegypti* and Ectotherm Development**

In *A. aegypti* the effects of water temperature on the development rate of aquatic stages and on adult size are well documented (Rueda et al., 1990; Tun-Lin et al, 2000). Temperature effects on development rate have been described by an enzyme kinetics model which assumes that development rate is controlled by a single rate-limiting enzyme that is denatured reversibly at high and low temperatures (Focks et al., 1993). This model has been shown to explain temperature effects on development rate in a wide range of ectotherms (Sharpe and DeMichele, 1977; Schoolfield et al., 1981), and is consistent with field and laboratory studies show that *A. aegypti* development rate increases linearly with temperature in the 15-35°C range, with a sharp increase in mortality below 20°C and above 30°C (Rueda et al., 1990, Tun-Lin et al., 2000).

Studies also show that adult size increases monotonically as temperature decreases in the range of 15 to 35°C (Rueda et al., 1990, Tun-Lin et al., 2000). The critical mass for pupation also varies inversely with temperature (Chambers and Klowden, 1990).

The enzyme-kinetics model forms the basis of temperature effects on vector development in the weather driven model, CIMSIM (Focks et al., 1993; Focks et al, 1995; Jetten and Focks, 1997). While the model predicts a monotonic increase in productivity in *A. aegypti* between 20 and 30°C, field studies do not reveal a consistent pattern. In two studies of productivity across individual containers in Puerto Rico (Barrera et al., 2006b) and Australia (Tun-Lin et al., 2000), temperature in the 22-30°C range was negatively associated with productivity, and both studies found that resource competition was the principal regulatory factor. Conversely, Favier et al. (2006) found that the number of pupae per positive container was correlated with seasonal temperature in an area of Southern Brazil with a large (~ 6°C) seasonal fluctuation in temperature.

The pattern followed by *A. aegypti* of faster development to a smaller final size with increasing temperature, dubbed the temperature-size rule (TSR) in ectotherms, is one of the most universal observations in biology (Atkinson and Sibly, 1997); yet the underlying developmental processes that generate this phenomenon in ectotherms remain controversial, and the issue has not been directly addressed in mosquitoes. For example, a mechanistic explanation is lacking for the widespread observation that while increased food and lower temperature both increase final ectotherm size, they have opposite effects on maturation time. VanderHave and deJong (1996), for example, postulated that the underlying mechanisms controlling cell division (mitosis) are different from those controlling differentiation (maturation rate), and thus environmental effects on body size may be reflected in the relative differences in cell size and number, According to

their model, which also assumes that temperature increases the activity of a single-rate limiting enzyme (Sharpe and deMichele, 1977), the TSR should apply if the process of cellular differentiation is more sensitive to temperature than cellular growth. This model predicts that size increases at lower temperatures should be generated by increases in cell size rather than cell number. Indeed, in a review of 12 studies on allometric growth in insects, 10 of which were carried out on *D. melanogaster*, it was concluded that, temperature generally affects body size through larger effects on cell size (Arendt, 2007). There is no reason to believe that these results apply in mosquitoes. However, they suggest that investigation of the allometry of body vis-à-vis cell size in mosquitoes may shed light on differential impacts of temperature and food intake.

An important question in vector borne disease is how mosquitoes will meet increased energy demands in a warming climate (Lafferty, 2009). However the assumption that size is determined exclusively through a single temperature-dependent enzyme, as used in prior models of *A. aegypti* immature dynamics (Focks et al, 1993), neglects potential interactions of temperature with energy acquisition and metabolism. An alternative group of models examines the TSR through the dynamic effects of temperature on catabolism and anabolism (Bertalanffy, 1960; Berrigan and Charnov, 1994; Perrin, 1995). Bertalanffy (1960) proposed a simple equation to outline the growth rates of organisms:

$$dw / dt = \eta w^m - \kappa w^n ,$$

where  $w$  is the mass of the organism,  $\eta$  and  $\kappa$  are the coefficients of anabolism and catabolism and  $m$  and  $n$  are the exponents that describe the weight dependence of anabolism and catabolism. Studies generally find that these exponents range between 0.5 and 1, indicating that anabolic and catabolic activity increases less than linearly with heightened weight. By assuming that the exponents  $m$  and  $n$  are constants, Perrin (1995) showed that the TSR can only arise if catabolic

coefficient is more sensitive than the anabolic coefficient to increasing temperature and cited empirical support in studies of fish. Moreover, by assuming that energy and mass are directly proportional, Angilletta and Dunham (2003) showed that, for the Bertalanffy-Perin explanation of the TSR to hold, growth efficiency must decrease with increasing temperature. Recently, Karl and Fischer (2008), using detailed laboratory experiments, showed that both food intake and assimilation efficiency are greater at lower temperatures in the copper butterfly, *Lycaena tityrus*. However, in a review of 97 studies across a range of taxa, it was found that growth efficiency was overwhelmingly *positively* correlated with temperature (Angilletta and Dunham, 2003). The authors conclude that empirical studies more strongly support the hypothesis that temperature effects emerge in the norm of development – that is, temperature modifies the trajectory of resource acquisition and usage through the exponents  $m$  and  $n$  in the Bertalanffy equation (Angilletta and Dunham, 2003). By affecting the exponents in addition to the coefficients of growth, temperature may cause the anabolic-catabolic balance to vary between the early and later stages of development. In *A. aegypti* this conclusion is supported by experimental work of, Rashed and Mulla (1989), who found that the food consumption rate per *A. aegypti* larva increased by 50% between 18°C and 31°C, suggesting that the Perin paradigm does not apply. Overall, these studies suggest that the way in which ectotherms allocate resources is a crucial aspect of the mechanisms determining how size and development rate change with increasing temperature. (Bochdanovits and De Jong, 2003; Kozłowski et al., 2004)

### **1.5 Hypotheses and Research Questions**

In dengue endemic areas of Colombia, climate warming may have complex interactions with larval ecology, as temperature directly affects the same principal outcomes as food intake and human behavior: survival, development time and growth. Because of its adaptation to low resource habitats, domestic *A. aegypti* in particular may exhibit unique interactions between

temperature, food consumption and growth. These biological interactions occur in a sociological context of household behaviors towards domestic container habitats, which in turn, may vary across climates. Given that *A. aegypti* populations thrive between 20-30°C, the temperature-specific response to increasing energy demands is likely to determine how this species responds to climate change. Since accumulation of energy stores are likely involved in the physiological process leading to *A. aegypti* maturation, which occurs sooner at increased temperature, it is unlikely that smaller body size at higher temperatures comes at the cost of energy storage. Ultimately, the impacts of climate change on dengue control will depend on how *A. aegypti* responds to the increased energetic demands of higher temperature (Lafferty, 2009), and how this response modifies the effects of water storage practices on *A. aegypti* production in specific ecological and social contexts.

In chapters 2-4 we explore the general hypothesis that temperature impacts on *A. aegypti* development are mediated by interactions with human behavior and feeding rate over the course of development. In particular, we address each of the following questions using either experimental temperature manipulations or a natural altitude gradient in Colombia:

1. How do household behaviors affect the rate of *A. aegypti* production and how are these relationships modified by the motivations for storing water in the context of an altitude-generated temperature gradient?
2. How do simultaneous manipulations of feeding rate and temperature differentially impact the size and number of epidermal cells in *A. aegypti* wings?
3. How do 2-degree deviations in temperature from 20 to 30°C impact tradeoffs between development rate, growth and starvation resistance in *A. aegypti*?

CHAPTER 2  
ECOLOGICAL LINKS BETWEEN WATER STORAGE BEHAVIORS AND *Aedes aegypti*  
PRODUCTION: IMPLICATIONS FOR DENGUE VECTOR CONTROL IN VARIABLE  
CLIMATES

**2.1 Introduction**

Throughout the world domestic vessels used for water storage are frequently the most abundant and productive habitats of immature stages of *Aedes aegypti* (L.), the principal vector of dengue fever (DF) and urban yellow fever (Southwood et al., 1972; Reuben et al., 1978; Barrera et al., 1993). This mosquito is uniquely influenced by human water storage, which, in turn, may affect indices of dengue vector abundance (Morrison et al., 2004; Barrera et al., 2006a; Hammond et al., 2007). Accordingly, understanding how specific patterns of water usage affect *A. aegypti* larval ecology may be a key step in identifying specific behaviors to target in dengue prevention interventions. (Elder and Lloyd, 2007)

Modifying human behavior for dengue control requires knowledge of local customs of water storage and how the resulting behaviors affect the processes that generate adult *A. aegypti*. While behaviors such as water use (Hammond et al., 2007), vessel emptying (Subra, 1983), cleaning (Chan et al., 1998) and covering with lids (Morrison et al., 2004) may reduce mosquito production, their implementation may not always be practical in particular water storage contexts. For example, frequent water usage may undermine the effects of lid placement and result in higher mosquito infestation rates in lidded vessels (Phuanukoonnon et al., 2005). Residents may be unwilling to empty vessels if water is stored primarily to compensate for unpredictable interruptions in piped water (Barrera et al., 1993). Among the unique processes that preimaginal mosquitoes experience in water storage vessels is a higher probability that aquatic stages are washed away by container emptying (Subra, 1983). Emptying, however, will only be effective in reducing *A. aegypti* production if it occurs before immature stages complete

development to adult. Thus, from an ecological perspective, emptying and associated behaviors may interact with conditions affecting development time, including temperature and food availability (Subra and Mouchet, 1984; Arrivillaga and Barrera, 2004; Barrera et al., 2006b).

Traditional entomological surveys are not suitable for correlating water storage practices with *A. aegypti* production. Respondents' self-perceptions, their desire to please vector control personnel, and the difficulties in quantifying temporally variable actions towards containers all limit the accuracy of self-reported behaviors in surveys. (Scrimshaw, 1990; Kasprzyk, 2005) Moreover, surveys with predetermined variables may not reveal the subjective experience of individuals in a particular social and physical context that influences their behavior (Golafshani, 2003). From a biological perspective, one-time pupal counts may also provide biased estimates of productivity across heterogeneous climates because higher temperature shortens the pupal stage, and pupae observed in warmer areas complete larval development faster than counterparts in colder areas (Christophers, 1960). Repeated household visits can address these difficulties (Subra, 1983), by averaging pupation across a fixed interval and characterizing human behavior through a combination of direct, repeated observation and open ended interviews (Trotter et al., 2001)

In Colombia, dengue viruses persist at altitudes from sea level to 1600m, where *A. aegypti* immature stages experience average temperatures ranging from 30-20°C in household containers. This temperature range is associated with larval developmental completion between 9-4 days under optimal feeding conditions (Rueda et al., 1990) and may cause the impact of human behaviors on pupal production to vary across cities at different altitudes. In this study we tested this hypothesis using a longitudinal (7 to 15 day) study of 235 households with larval infested water storage vessels in three Colombian cities 5, 950 and 1550 meters above sea level

(msl). In particular, we characterized motivations for household water usage and the effects of water storage practices on the rate of *A. aegypti* production in order to suggest the behaviors most likely to reduce *A. aegypti* production in water storage vessels in each city.

## 2.2 Materials and Methods

### 2.2.1 Study Area

This study was conducted in two DF endemic neighborhoods in each of the cities Armenia (1550 msl), Bucaramanga (950 msl) and Barranquilla (5 msl). All six neighborhoods had a socio-economic ranking of 2 (on a 1 to 6 scale of each municipal planning department), characterized by low-income, planned housing units. In 2007 all six neighborhoods had a stable piped water supply with occasional interruptions. Routine vector control in 2007 varied across cities: in Armenia there were no known interventions; in Bucaramanga, adulticides were applied in homes within 100 m of dengue cases and larvicides were applied to storm drains; in Barranquilla there was a city-wide mass communication program to promote cleaning, scrubbing and lid placement in domestic vessels.

Data collection in each city was conducted in January, June and October 2007. Ten-year mean ambient temperatures (°C) and relative humidity (%) measured in airport weather stations for each of these respective months are: Armenia – 20.3 (79.7%), 20.3 (81.4%), 20.0 (82.7%); Bucaramanga – 23.3 (81.4%), 23.3 (84.9%), 22.9 (85.3%); Barranquilla – 26.8 (78.4%), 28.3 (81.4%), 27.6 (85.4%) (Institute of Environmental Studies of Colombia, 2009). It should be noted, however, that the weather stations for Armenia and Bucaramanga differ in altitude as compared to the study neighborhoods, which causes temperature to differ between airports and our study areas (see below). Mean cumulative monthly rainfall (mm) for January, June and October is the following: Armenia – 144.0, 183.7, 278.8; Bucaramanga – 78.3, 99.4, 93.6; Barranquilla – 1.7, 80.2, 152.8 (Institute of Environmental Studies of Colombia, 2009). Seasonal

climate patterns are not consistent across cities although January is the driest of the three survey periods in all three.

Based on their high rates of dengue persistence (2004 to 2006) neighborhoods were selected as part of a separate study of human ecology and *A. aegypti* production (not reported here). This study consisted of tri-annual surveys of 7159 households (35-50% of each neighborhood) and their water-holding containers, carried out in the same months as the present study. Preliminary 2007 data from this study showed that water storage vessels accounted for over 90% of all *A. aegypti* pupae in each city, with Breteau Indices ranging 18.3-28.1 infested vessels/100 premises inspected in Armenia, 11.5-24.4 in Bucaramanga and 6.7-13.2 in Barranquilla.

### **2.2.2 Container Selection**

For the present study we selected actively-filled water storage vessels found infested with *A. aegypti* larvae or pupae in the triannual surveys described above and carried out visits every other day over 10-15 days after detecting the vessel. In each survey period we sought authorization to conduct the study in all premises with a larval or pupal infested vessels within a subset of 240-360 houses in Bucaramanga, 360-480 houses in Armenia and 600-720 houses in Barranquilla. Sampling effort was determined based on available manpower in each city. Houses with more than one infested actively filled water storage vessel (found rarely and only in Barranquilla) were excluded from the study in order avoid the added complication of assessing potential variation in behavior towards multiple vessels, and no houses were repeatedly followed in different study periods. Residents were informed that participation involved receiving a visit every two days in order to count all mosquito pupae and to query their container use in the previous 48 hours. The minimum inclusion criterion was at least three visits over a minimum

seven-day interval. Containers not surveyed on two consecutive visits because residents were absent at the time of our visit were eliminated from the study.

### 2.2.3 Physical Description of Houses and Study Vessels

In houses of all six neighborhoods water storage vessels were located in a concrete wash area in the *patio* – an enclosed semi-roof covered area towards the rear of the house. In Barranquilla, patios range from 5 up to 30 m<sup>2</sup>. This sharply contrasts with Bucaramanga and Armenia, where patios range 1-5 m<sup>2</sup>. All vessels studied in Bucaramanga and Armenia were at least partially roof-covered and received little or no sunlight and rainfall. In Barranquilla, the warmest city, 46% of study vessels were classified as without roof cover but received limited direct sunlight due to their proximity to the houses.

Ninety-two percent of study vessels in Armenia and Bucaramanga were *lavaderos*, or washbasins and faucet with a capacity of 100-150 L, an opening of approximately 800 cm<sup>2</sup> and an attached scrubbing board, elevated approximately 1m above the floor (Figure 2A). *Lavaderos* have a removable drain to easily empty water. In Barranquilla homes were not constructed with a built in washbasin, so residents obtained or constructed water storage vessels. Combined with the larger *patio* size, this resulted in larger variability in Barranquilla vessel types, including 500 L cement tanks, 200 L metallic and plastic drums, 20-50 L plastic buckets/jugs and occasional > 1000 L cement *albercas* (pools) (Figure 2B). However, because we exclusively studied actively filled water storage vessels, observed water volume varied on a daily basis and was almost entirely dependent on household use patterns, given high shade cover and thus low evapotranspiration.

## **2.2.4 Data Collection**

### **2.2.4.1 Water temperature**

Digital temperature loggers (Embedded Data Systems®) were placed in water storage vessels in each neighborhood over 10-15 days during the June 2007 surveys. Loggers were placed in households where we felt there was minimal risk of loss or theft; there was no indication of any systematic differences in water temperatures between these and our study households. Eight loggers were placed in Bucaramanga, and 12 in Barranquilla and Armenia, respectively. ANOVA and pairwise T-tests were employed to compare mean temperatures from hourly recordings in each container across cities. Representative of conditions in study vessels, all Bucaramanga and Armenia loggers were placed in roof-covered *lavaderos*, whereas 50% of the Barranquilla loggers were placed in plastic and metal drums partially exposed to sunlight and the other 50% in large cement basins. All vessels contained at least 20 L of water,

### **2.2.4.2 Entomological surveys**

All pupae were collected upon discovery to avoid the possibility of re-counting on successive visits, and all mosquito immatures were eliminated at the end of the study. Pupae were returned to the public health laboratory of each municipal health department and allowed to emerge to adult in order to confirm species identification. For each vessel the mean two-day rate of production was defined as the total number of *A. aegypti* pupae collected over the study period divided by the number of observations (visits in which entomological survey was carried out).

### **2.2.4.3 Human behavioral assessments**

Behaviors were characterized through semi-structured interviews conducted in each visit with a household member that interacted frequently with vessels. On each visit we carried out an informal interview of residents, allowing them to express the reasons for using stored water and the nature of the use. Studies show that such inductive data collection reduces self-reporting

biases associated with structured survey with pre-determined variables that subjects may subjectively interpret (Golafshani, 2003). Ethnographic narratives (Caprara et al., 2009) were employed in order to qualitatively describe the motivations underlying water storage behaviors in each city.

Three behaviors were consistently recorded for each container-household: whether the water was used (self-reported) or emptied (self-reported triangulated with observation) in the prior 48 h, and whether the vessel was lidded (observation triangulated with self-reporting).

### **2.2.5 Data analysis**

Given the stochastic nature of human behaviors and short follow-up time (7-15 days) per vessel, emptying interval and usage were grouped into discrete levels, using biologically and sociologically relevant cutoffs. Water usage (extraction for domestic use) was classified into the following categories of decreasing use: (1) every visit, (2) intermittently or (3) never. Increasing water emptying interval was classified as: (1) < 7 days between occurrences, corresponding roughly to average larval development times above 24°C under optimal food conditions (Rueda et al., 1990); (2) 7-15 days between occurrences and (3) no emptying recorded in the study. Usage of a container lid (covering entire vessel opening) was dichotomized. Spearman rank correlations and contingency table ( $\chi^2$ ) analyses were used to correlate behaviors in each city.

#### **2.2.5.1 Explanatory variables entered in model**

Because of the potentially complex causal pathways between behavior and physical variables such as container location, volume, debris, microclimate and others (see discussion) we limited independent variables to usage (categorical), emptying (categorical), lid placement, survey period, city and the interaction terms Barranquilla\*emptying7-15days and Armenia\*emptying 7-15days. Based on preliminary data inspection, we included these interactions in order to test for a non-linear effect of emptying on production in the vicinity of

the time to pupation, as minimum larval development period (under optimal laboratory conditions unlikely to be met in the field) is 8 and 4 days at the temperatures of Armenia (21.9°C) and Barranquilla (28.3°C), respectively (Rueda et al., 1990).

### 2.2.5.2 Model building and selection

Based on the distribution of the dependent variable, mean pupae/two-day interval/vessel, we used negative binomial regression in STATA 8.0 (StataCorp. 2001. Statistical Software: Release 8.0. College Station, TX: Stata Corporation). A “saturated” multivariate model was constructed using the explanatory variables listed above. Potential variation in the aggregation parameter ( $\alpha$ ) [as defined in variance equation for the negative binomial model (Eq. 2-1)] across city (Table 2-1) and behavioral (Table 2-2) strata, was accounted for by employing the generalized negative binomial regression (*gnbreg*) command. This procedure tested whether specification of  $\alpha$  across one of these groups improved the saturated model by parameterizing the equation (2-2). All variables were entered into Equation 2-2 as dichotomous (dummy variables for each category in ordered variables). The alpha parameterization that most improved the overall saturated model was then maintained in deriving best-fitting models.

$$s^2 = \bar{x} + \alpha \bar{x}^{-2} . \tag{2-1}$$

$$\ln \alpha = a_0 + a_1 X . \tag{2-2}$$

We selected the best fitting model using the Akaike Information Criterion (AIC), defined as,

$$AIC = 2k - 2LL , \tag{2-3}$$

where  $k$  is the number of free parameters and LL is the log-likelihood (Hobbs and Hilborn, 2006). The minimum AIC model was selected among the 20 combinations of main effects and

interaction terms specified above with the highest likelihoods. Uncertainty in model selection was evaluated using AIC weights ( $w_r$ ) defined as,

$$w_r = e^{-2\Delta_r} / \sum_{i=1}^R e^{-2\Delta_i} , \quad (2-4)$$

where  $\Delta_r$  is the difference between the AIC value of model  $r$  and the lowest AIC model, and  $R$  is the total number of models considered. The index  $w_r$  can be interpreted as an indicator of the odds that model  $r$  would emerge as the best model among the attempted models given a different dataset (Hobbs and Hilborn, 2006).

### 2.2.5.3 City-specific models

City-specific models were constructed using the same process and alpha parameterization as the overall model. Potential non linear-effects of emptying interval in the vicinity of larval development period were explored by inserting emptying < 7 days and emptying 7-15days as dummy variables for comparison against non-emptied vessels, instead of the categorical approach used in the overall and univariate analyses.

## 2.3 Results

### 2.3.1 Study Containers, Water Storage Practices and *A. aegypti* Production

Overall, 235 water storage vessels infested with *A. aegypti* larvae or pupae were included in the study. Mean production was similar in Armenia and Barranquilla, but lower in Bucaramanga (Table 2-1). A similar proportion of vessels across cities had missing data due to absence of residents. Mean water temperatures coincided with altitude and were significantly different between cities ( $F_{(2)} = 212.3$   $p < 0.001$ ). There were also significant pairwise differences between the two colder cities, even after a Bonferonni adjustment of the significance level to  $\alpha/n$ , or 0.0025 ( $t_{\text{bucaramanga-armenia}} = 6.84$   $p < 0.001$ ). Variation in manpower, infestation levels, resident cooperation and follow-up success all contributed to the observed differences in sampling effort

across cities. Overall, 5.3 observations were made per container, corresponding to a mean follow up interval of roughly 11 days (Table 2-1).

Water in 83.4% of the vessels was extracted for domestic use at least once during the follow-up period and 80.4% lacked a lid (Table 2-2). Forty-two percent of the vessels were never emptied and these produced 67.2% of the total *A. aegypti* pupae recovered; 92% of total pupae were produced in vessels emptied at least every 7 days. Production rate was similar across water use groups and was lower in lidded vessels (Table 2-2). Follow-up interval was similar across behavioral strata.

### **2.3.2 Qualitative Assessment of Behavior and Motivations**

We observed strong relationships between water storage behavior and container structure. In Armenia and Bucaramanga, residents stored water primarily for the convenience of using the easily accessible washbasin instead of tap water to wash clothes and, secondarily, for cleaning floors, flushing toilets, watering plants, or in case of interruptions in piped service. Potential interruption in piped supply was the major factor influencing water storage in Barranquilla, however no clear interactions between interruptions in piped service and water storage practices were observed. In Barranquilla elderly residents, who lived most of their lives without piped water, were often those that had the custom of storing water “just in case.” Residents specifically mentioned an interruption in piped service as the reason for water use on only 11 occasions in 10 different houses (6 in Barranquilla and 4 in Bucaramanga). In all cities the large majority of vessels followed contained exclusively tap water, and no vessel followed was exclusively rainwater.

Container structure influenced lid placement. Metallic and plastic drums (200 L), observed only in Barranquilla, often had tailor-made lids, as did 1000 L cement/plastic tanks (Figure 2-2B). In some instances residents in Barranquilla improvised lids on 1000 L permanent

basins with household materials such as wooden boards, plastic bags, cardboard or roof tiles. *Lavaderos* such as Figure 2-2A were seldom lidded.

### **2.3.3 Quantitative Assessment of City-Specific Behaviors**

Container lid placement was less frequent in Armenia than in the other two cities (Table 2-3). On average water was used less frequently in Barranquilla than in Armenia and Bucaramanga, whereas the emptying interval in Barranquilla was almost double that of the Andean cities (Table 2-3). Twenty seven percent of vessels in Barranquilla were never used during the observation period, as compared to 6.9% and 9.3% in Armenia and Bucaramanga, respectively. Water usage and emptying were significantly correlated ( $p < 0.05$ ) in all 3 cities (Table 2-3), although the correlation was much higher in Barranquilla (Table 2-3).

In all three cities, containers surveyed in the dry season (January 2007) had a notably higher mean interval between emptying events (Table 2-4). In Barranquilla, this also coincided with decreased usage, whereas there was no apparent pattern of water usage across surveys in Bucaramanga and Armenia. Production rate, particularly in Armenia and Barranquilla, was also higher in the dry season survey (Table 2-4).

### **2.3.4 Effects of Water Storage Behaviors and City on *A. aegypti* Production**

The mean rate of mosquito production across vessels was well approximated by a negative binomial distribution ( $\alpha = 3.35$ ,  $\chi^2_{(1)} = 6505$ ,  $p < 0.01$ , rejecting the null hypothesis that  $\alpha = 0$  in Equation 2-2). Among univariate models, increasing emptying interval best described the variation, followed by lid placement and survey period (Table 2-5). Vessels in Bucaramanga were associated with lower pupation as compared to the other two cities (negative coefficient in Table 2-5). Decreasing use frequency, Armenia and Barranquilla had higher AICs than the intercept only model (Table 2-5). In univariate models, each of the three cities was dummy

coded as a dichotomous variable in order to compare the effect of each individually against the data from the other two.

The log-likelihood (LL) of the standard *nbreg* saturated model with all behaviors, interaction terms and city effects was -694.3 (AIC=1404.6) as compared to -690.9 (AIC=1399.8) when *gnbreg* was employed using the equation  $\ln(\alpha) = 0.86 + 0.67(\text{emptying} < 7\text{days})$ , indicating that the data were best described when the model took into account increased aggregation of production in containers emptied < 7days. Specification of  $\alpha$  for other behaviors, categorical emptying interval, or cities generated smaller or no improvements in AIC as compared to the model with unspecified alpha; therefore all subsequent multivariate models (Tables 2-6 and 2-7) employed this parameterization of  $\alpha$ .

Less frequent emptying was associated with increased production. Barranquilla\*7-15day emptying had a positive and Armenia\*7-15day a negative effect, with the latter having a 0.07 probability of being selected in the best-fitting model based on AIC weights ( $w_i$ ) (Table 2-6), whereas the former was the strongest predictor in the best fitting model. In the Barranquilla only model, emptying < 7days had a strong negative effect on production (Table 2-7), whereas emptying 7-15 days did not improve the description of the data (Table 2-6). In Bucaramanga, inclusion of both emptying intervals improved the prediction of production rate as compared to non-emptied vessels (Table 2-6), but emptying < 7 days had a 3-fold greater effect in reducing production than emptying 7-15 days (Table 2-7). In Armenia emptying < 7 and 7-15 days had a similar effect in reducing production (Table 2-7). In Barranquilla production rate is similar in vessels not emptied and emptied 7-15 days (Figure 2-3), whereas in Armenia, production is similar in vessels emptied <7 days and emptied 7-15 days. In Bucaramanga, production appears to linearly increase with emptying category (Figure 2-3).

Water usage frequency did not significantly improve the model fits of the overall data (Table 2-6). Decreased usage was slightly associated with reduced production in the best-fitting models for Armenia and Barranquilla (Table 2-7), but the same models without the usage term had large  $w_r$  values (0.29 and 0.35, respectively) indicating a relatively high probability that water usage would not appear in the best-fitting model in another data set (Table 2-6).

Lid placement was associated with reduced production overall, but only improved model fits for Barranquilla (Tables 2-6 and 2-7). Including survey period improved model fits for the overall data set, with  $w_r = 0.70$  as compared to  $w_r = 0.10$  when this variable was omitted (Table 2-6). Survey period remained in the best-fitting models of Barranquilla and Bucaramanga, but with opposite effects (Table 2-7).

## 2.4 Discussion

Human behaviors generate unique habitats for mosquito development in domestic water storage containers. Since recognizing important behaviors is a key step in designing community-based *A. aegypti* control strategies (Elder et al, 1998; Elder and Lloyd, 2007), there is a need to understand how water storage practices interact with human and mosquito ecology. For dengue prevention policy, the results of this study suggest that in defining community-based interventions, vector control programs should include assessment of local ecological factors affecting larval development rate, prevalent water storage behaviors, and how the latter interacts with environmental features such as container structure and seasonality.

Our results are consistent with a non-linear relationship between pupal production and household water emptying interval, whereby the impact of emptying *A. aegypti* habitats every 7-15 days on reducing production may decrease in cities with increasing mean temperature within the 20-30°C range. In Barranquilla (29.3°C) the effect of emptying on production was strongly

modified upward in the 7-15day interval, whereas in Armenia there was a weak modification towards lower production in this interval (Table 2-6). Moreover, in Armenia, the effects of emptying < 7 and 7-15 days as compared to non-emptied vessels were approximately the same, whereas in Barranquilla 7-15day emptied vessels had similar productivity to those that were never emptied; Bucaramanga (23.9°C) demonstrated significant increases in productivity across the 3 emptying levels (Figure 2-3, Table 2-7). Thus, the impact of human emptying on vector production appears to be dependent on city-specific ecological factors such as temperature that may affect development rate, such that small changes in emptying interval may have a large impact on *A. aegypti* output. In Australia human adaptation to increased drought has been shown to drive *A. aegypti* infestation more than the direct temperature effects (Beebe et al., 2009). Our results suggest that emptying frequency was lower in the dry season survey which would indicate that climate change-induced droughts in Colombia may increase *A. aegypti* production via human adaptation. However, study households were not selected randomly nor revisited in different seasons. Future studies should focus on human perceptions of climate variability, seasonality and long term climate change, and how these affect container interactions. We suggest that temperature-driven models of *A. aegypti* production (Focks et al., 1993, Jetten and Focks, 1997) could be improved by incorporation of human behavioral dynamics.

We found that container structure and location in the household environment shapes the underlying motivations for water storage and behavior towards vessels. The cement washbasin, present in virtually every house in Armenia and Bucaramanga, allowed residents to handwash clothes using stored, instead of tap, water. Accordingly, over 90% of residents in Armenia and Bucaramanga used stored water in comparison to only 63% in Barranquilla. Households that frequently used vessels were more apt to regularly empty water, but frequent use may make lid

usage more cumbersome (Phuanukoonnon et al., 2005). In contrast, emptying was less frequent in households that store water not for regular use. After controlling for emptying frequency, however, increased usage of wash basins was not associated with production or may have slightly increased it in Armenia and Barranquilla – perhaps through increased egg hatching stimuli through frequent fluctuations in water level. In Barranquilla, the lack of a built-in storage vessel compelled residents to actively obtain vessels to store water, largely in case of interruptions in piped service, thereby generating a stronger correlation between emptying and use (Table 2-3). Moreover, greater distance between stored water and washing area reduced the use of stored water for cloth washing. We suggest that less frequent use in Barranquilla, combined with the structure of storage drums and possibly community education programs, allowed lid placement to be more frequent and effective in reducing production as compared to the other two cities. (Table 2-3 and Table 2-7).

For vector control, understanding interactions between larval ecology and human behavior is important for negotiating intervention strategies with communities so as to maximize the sustainability of community-wide behavior modification (Elder and Lloyd, 2007). For example, in Armenia emptying at least every two weeks is sufficient to reduce vector output, whereas a more intense promotional campaign would be required in Bucaramanga as the decrease achieved by emptying once per week is more than 3-fold that obtained upon emptying once every two weeks (Table 2-7). In Barranquilla promotion of emptying once a week may be useful in frequently used vessels, but other strategies, such as lid placement, should be considered for households that do not regularly use stored water. In the dry season, particularly in Barranquilla, people may be more reluctant to empty vessels, suggesting a role for seasonal larviciding programs. We conclude that in Colombia, vector control programs could be

optimized by specifying the emptying frequency required to reduce *A. aegypti* production in a particular altitude or season and promoting alternative behaviors when the underlying motivation for water storage, as influenced by local container structure or reliability of water service, does not permit fulfillment of this objective.

We found that container emptying drives variation in the both mean and aggregation of *A. aegypti* production across water storage vessels. Fifty-eight percent of the total production was concentrated in 7% of the vessels, of which only one was emptied more than once every seven days. This result bears a remarkable resemblance to findings of Subra (1983) who followed 53 vessels in a small Kenyan village over 63 days. Moreover, using a generalized model, we show that aggregation of pupal production was higher in vessels emptied  $< 7$  days, most likely because of a large number of frequently emptied vessels that never produced pupae. *A. aegypti* pupae are usually highly aggregated in cross sectional surveys, and our results indicate that the dynamics of emptying may generate this consistent pattern in which the majority of larval infested vessels in urban areas produce few or no pupae (Focks and Chadee, 1997; Getis et al., 2003; Morrison et al., 2004; Barrera et al., 2006a). In addition to flushing out immatures, emptying may reduce larval food, a well documented regulator of pupal production in water storage vessels (Subra and Mouchet, 1984, Arrivillaga and Barrera, 2004, Barrera et al., 2006b).

Human perceptions and socio-cultural factors interact with the physical environment to define human motivations. These motivations determine human-container interactions, including the frequency and intensity of water usage and replenishment, the time water goes unperturbed without emptying or cleaning, location, lid placement, etc. These processes, in turn, determine key *A. aegypti* habitat features such as the quality of vessel construction, location (shade, sun, rainfall), exposure and accumulation of detritus, and the dynamics of water volume – which

ultimately shape the proximal ecological processes that regulate larval development including density dependent and independent intraspecific resource competition, microclimate, oviposition, egg accumulation, egg hatching frequency, etc.

In this study we focused on directly associating human behaviors with *A. aegypti* pupal output and chose to exclude intermediate variables such as daily volume, container size, location and structure from the model selection processes. Because of the complex and multiple causal pathways through which human behavior, container structure and proximal physical variables may interact, and given limited sample size, we chose to exclude physical variables. For example, in Barranquilla, with a relatively stable water supply and no built-in washbasin, a frail person may chose to store water in easily maneuverable and sealable 20 L buckets, instead of a large 200 L plastic tank, given the more difficult maintenance and that water is only used in emergency situations. Other residents may choose a large 1000 L tank for easier access to water, but due to intermittent usage, it may be placed in an unobtrusive location, increasing exposure to sunlight, rainfall and nutrient entrance. Alternatively, for a renter, such a vessel may condition decreased usage because of the distance between the vessel and the washing area, thereby reversing causality between container structure and water storage behavior. In certain cases unrecorded physical variables, could have modified the impacts of human behavior – for example, container texture and contour may affect how efficiently larvae or food particles are removed when permanent basins are emptied by removing the drain. Indeed, while vessels in Armenia and Bucaramanga were virtually identical in size, material and use patterns (Figure 2-2, Table 2-3), Bucaramanga washbasins tended to have smoother finishing, which may have contributed to the observed lower production (Table 2-1). Moreover, our inductive behavior analysis, employed to minimize the chances that residents would modify behaviors in response to

our surveys, impeded quantification of other sociological variables such as interruptions in piped water supply, container cleaning/scrubbing or socio-economic factors.

Nonetheless this study suggests key variables that may interact with human behavior including container structure, contour, location, human demographics, , temperature and seasonal variation in water service . We show that *A. aegypti* production and human behavior are a coupled human-ecological system (Wilcox and Colwell, 2005; Liu et al., 2007); research on dengue prevention in variable climates must focus the shared dependency of households and *A. aegypti* immatures on domestic vessels and their water.

Table 2-1. Water storage containers (infested with *A. aegypti* larvae) studied, follow-up interval, missing data and pupation rates

| City         | Vessels studied (mean no. observations/vessel) | Mean 2-day <i>A. aegypti</i> pupal count ( $\alpha^*$ ) | Containers with missing data ** (% total) | Mean water temperature (°C) (range) |
|--------------|--|---|---|-------------------------------------|
| Armenia      | 77 (5.6)                                       | 13.9 (4.6)  | 22 (26%)                                  | 21.9 (21.0-23.3)                    |
| Bucaramanga  | 47 (5.8)                                       | 7.2 (3.2)   | 16 (30%)                                  | 23.9 (22.8-25.5)                    |
| Barranquilla | 111 (4.9)                                      | 12.0 (2.8)  | 30 (25%)                                  | 29.0 (27.2-31.0)                    |
| OVERALL      | 235 (5.3)                                      | 11.6 (3.5)  | 68 (26%)                                  |                                     |

Note:

\* Aggregation parameter ( $\alpha$ ), defined in the negative binomial formulation:  $s^2 = \bar{x} + \alpha \bar{x}^{-2}$ , where  $\alpha=0$  indicates that a Poisson model is adequate for describing the distribution of production across vessels

\*\* Residents were absent on one visit and container was not surveyed

Table 2-2. Emptying, usage, lidding and *A. aegypti* production in study vessels

|                               | No. vessels (avg. no obs/vessel) | Mean <i>A. aegypti</i> pupae/ 2-day interval ( $\alpha^*$ ) | Percent total vessels | Percent total <i>A. aegypti</i> pupae |
|-------------------------------|----------------------------------|---|-----------------------|---------------------------------------|
| Max. days between emptying    | <7                               | 66 (5.3)  | 28.1%                 | 8.0%                                  |
|                               | 7-15                             | 70 (5.9)  | 29.8%                 | 24.8%                                 |
|                               | Not emptied                      | 99 (4.9)  | 42.1%                 | 67.2%                                 |
| Max. days between water usage | ≤2days                           | 92 (5.3)  | 39.1%                 | 40.1%                                 |
|                               | 3-15 days                        | 104 (5.5)   | 44.3%                 | 41.0%                                 |
|                               | Not used                         | 39 (4.8)  | 16.6%                 | 18.9%                                 |
| Lid placement                 | No                               | 189 (5.2)   | 80.4%                 | 91.4%                                 |
|                               | Yes                              | 46 (5.7)  | 19.6%                 | 8.6%                                  |

Note:

\* Aggregation parameter ( $\alpha$ ), defined in the negative binomial formulation:  $s^2 = \bar{x} + \alpha \bar{x}^{-2}$ , where  $\alpha=0$  indicates a random production rate across vessels.

Table 2-3. City-wide differences in patterns of use, emptying and lidding of vessels

|  | Armenia             | Bucaramanga         | Barranquilla          |
|--|---------------------|---------------------|-----------------------|
| Containers with lids (% total)   | 6 (8.0%)            | 11 (23%)            | 29 (26%)              |
| Avg. water emptying interval (SD)  | 8.5 (9.8)           | 10.2 (8.8)          | 13.1 (10.4)           |
| Avg. water usage interval (SD)   | 2.5 (6.6)           | 2.6 (5.6)           | 4.7 (5.4)             |
| Containers never used during study (% total)   | 5 (6.9%)            | 4 (9.3%)            | 30 (27%)              |
| Spearman's Rho, d.f., correlating usage vs. emptying interval (p-value)                          | 0.31, 47<br>(0.005) | 0.32, 77<br>(0.029) | 0.61, 111<br>(<0.001) |
| Contingency table $\chi^2_{(2)}$ Usage (always, intermittent, never) vs. lid placement (p-value) | 3.4<br>(0.33)       | 6.7<br>(0.08)       | 2.9<br>(0.40)         |
| Contingency table $\chi^2_{(2)}$ Emptying (<7days, 7-15days, never) vs. lid placement (p-value)  | 5.0<br>(0.08)       | 0.49<br>(0.78)      | 1.5<br>(0.47)         |

Table 2-4. Patterns of use, emptying and rate of *A. aegypti* production across survey periods

| City         | Survey period in 2007<br>(no. vessels followed) | Container use<br>interval (s.e.) | Container<br>emptying interval<br>(s.e.) | Mean 2-day rate<br>of <i>A. aegypti</i><br>production (s.e.) |
|--------------|---|----------------------------------|--|--|
|              | January (24)                                    | 2.4 (6.6)                        | 12.6 (9.6)                               | 23.1 (48.2)  |
| Armenia      | July (18)                                       | 2.4 (8.5)                        | 8.0 (12.7)                               | 13.3 (15.5)  |
|              | Oct-Nov (35)                                    | 2.6 (6.0)                        | 7.20 (9.5)                               | 7.8 (26.1)   |
|              | January (13)                                    | 2.7 (5.1)                        | 28.4 (14.8)                              | 8.6 (15.5)   |
| Bucaramanga  | July (21)                                       | 2.3 (6.6)                        | 7.9 (7.3)                                | 5.6 (15.0)   |
|              | Oct-Nov (13)                                    | 3.1 (5.3)                        | 8.8 (11.9)                               | 8.3 (6.9)  |
|              | January (23)                                    | 5.8 (4.7)                        | 39.4 (17.0)                              | 28.9 (46.0)  |
| Barranquilla | July (51)                                       | 4.8 (5.9)                        | 10.0 (9.6)                               | 6.5 (9.7)  |
|              | Oct-Nov (37)                                    | 4.1 (5.4)                        | 13.2 (11.2)                              | 9.1 (28.2)   |

Table 2-5. Univariate associations between water storage behaviors and city and mean *A. aegypti* pupal production in infested vessels in order of log-likelihood (LL)

| Variable          | Coefficient (95% CI) | $\Delta$ LL | $\Delta$ AIC |
|-------------------|----------------------|-------------|--------------|
| Emptying interval | 0.84 (0.54,1.15)     | 0           | 0            |
| Lid placement     | -1.19 (-1.8,-0.62)   | -10.16      | 20.33        |
| Survey period     | -0.45 (-0.73, -0.18) | -10.51      | 21.03        |
| Bucaramanga       | -0.58 (-1.17, 0.015) | -14.17      | 28.35        |
| Armenia           | 0.27 (-0.23, 0.77)   | -15.23      | 30.47        |
| Use category      | 0.056 (-0.15, 0.26)  | -15.65      | 31.43        |
| Barranquilla      | 0.061 (-0.41, 0.53)  | -15.76      | 31.53        |
| Intercept only    | 2.45 (2.22, 2.69)    | -15.79      | 29.59        |

Table 2-6. Multivariate models of mean rate of *A. aegypti* pupal production, using generalized negative binomial regression<sup>1</sup> for overall data and within cities

| Dataset      | Explanatory variables (sign of effect)  | LL      | AIC ( $w_r$ )                     |
|--------------|---|---------|-----------------------------------|
| Total        | Emptying interval (+), decreasing use (+), lid (-), emptying7-15 *Barranquilla (+), empty7-15*Armenia (-), Armenia (+), Barranquilla (+), survey period | -690.91 | 1399.84<br>( $7 \times 10^{-5}$ ) |
|              | Emptying interval (+), lid (-), emptying7-15*Barranquilla (+), Armenia (+), survey period   | -691.52 | 1395.03<br>(0.70)                 |
|              | Emptying interval (+), lid (-), emptying7-15*Barranquilla (+), Armenia (+)  | -692.99 | 1395.97<br>(0.10)                 |
|              | Emptying interval (+), lid (-), emptying7-15 days*Barranquilla (+), empty7-15days*Armenia (-), Armenia (+), survey period                               | -691.10 | 1396.20<br>(0.07)                 |
| Armenia      | Emptying<7days (-), emptying7-15days (-), decreasing use (-), lid (-), survey period  | -216.98 | 445.96<br>(0.001)                 |
|              | Emptying<7days (-), emptying7-15days (-), decreasing use (-)  | -217.44 | 442.89<br>(0.48)                  |
|              | Emptying<7days (-), emptying7-15days (-)  | -218.57 | 443.13<br>(0.29)                  |
|              | Decreasing use (+), emptying7-15days (-)  | -218.76 | 443.53<br>(0.13)                  |
| Bucaramanga  | Emptying<7days (-), emptying7-15days (-), decreasing use (+), lid (-), survey period  | -116.54 | 245.09<br>(0.001)                 |
|              | Emptying<7days (-), emptying7-15days (-), survey period   | -116.91 | 241.82<br>(0.93)                  |
|              | Emptying<7days (-), emptying7-15days (-)  | -118.77 | 243.53<br>(0.03)                  |
|              | Emptying<7days (-)  | -119.78 | 243.55<br>(0.03)                  |
| Barranquilla | Emptying<7days (-), emptying7-15days (-), decreasing use (+), lid (-), survey period <sup>2</sup>   | -343.33 | 698.65<br>(0.02)                  |
|              | Emptying<7days (-), lid (-), survey period, decreasing use (-)  | -343.52 | 697.05<br>(0.62)                  |
|              | Emptying<7days (-), survey period, lid (-)  | -344.66 | 697.33<br>(0.35)                  |

Note: For dataset, the saturated model is given first, followed by the 3 models with the lowest AIC (bold indicates best-fitting models) out of the 20 highest likelihood models considered for each data set.

<sup>1</sup>Using the *gnbreg* command in STATA 8.0 in which the aggregation of production across vessels was parameterized according to  $\ln \alpha = a_0 + a_1(\text{emptying} < 7\text{days})$

\* In Barranquilla the saturated model was the 3<sup>rd</sup> best fitting model.

Table 2-7. Overall and city-specific best fitting models of household behaviors and *A. aegypti* pupal production

| Model (Chi-2 comparison to intercept-only model)    | Explanatory variables            | Coefficient (95% CI) |
|---|----------------------------------|----------------------|
| Overall<br>( $\chi^2_{(5)}=39.5$ , $p<0.001$ )      | Emptying interval (linear trend) | 0.82 (0.51, 1.14)    |
|   | Lid placement                    | -0.65 (-1.25,-0.05)  |
|   | Barranquilla*emptying 7-16 days  | 0.93 (0.22, 1.64)    |
|   | Armenia                          | 0.47 (-0.05, 0.98)   |
|   | Survey period                    | -0.26 (-0.56, 0.03)  |
| Armenia<br>( $\chi^2_{(3)}=13.85$ , $p=0.003$ )     | Emptying interval < 7 days       | -1.26 (-2.60, 0.11)  |
|   | Emptying interval 7-16 days      | -1.32 (-2.30,-0.35)  |
|   | Decreasing use                   | -0.60 (-1.30, 0.08)  |
| Bucaramanga<br>( $\chi^2_{(3)}=14.82$ , $p=0.002$ ) | Emptying interval < 7 days       | -4.80 (-6.7, -3.00)  |
|   | Emptying interval 7-16 days      | -1.55 (-2.9, -0.21)  |
|   | Survey period                    | 0.78 (-0.06, 1.60)   |
| Barranquilla<br>( $\chi^2_{(4)}=27.1$ , $p<0.001$ ) | Emptying interval < 7 days       | -1.65 (-2.5, -0.83)  |
|   | Lid                              | -0.98 (-1.6, -0.28)  |
|   | Decreasing use                   | -0.26 (-0.60, 0.08)  |
|   | Survey period                    | -0.53 (-0.93,-0.12)  |

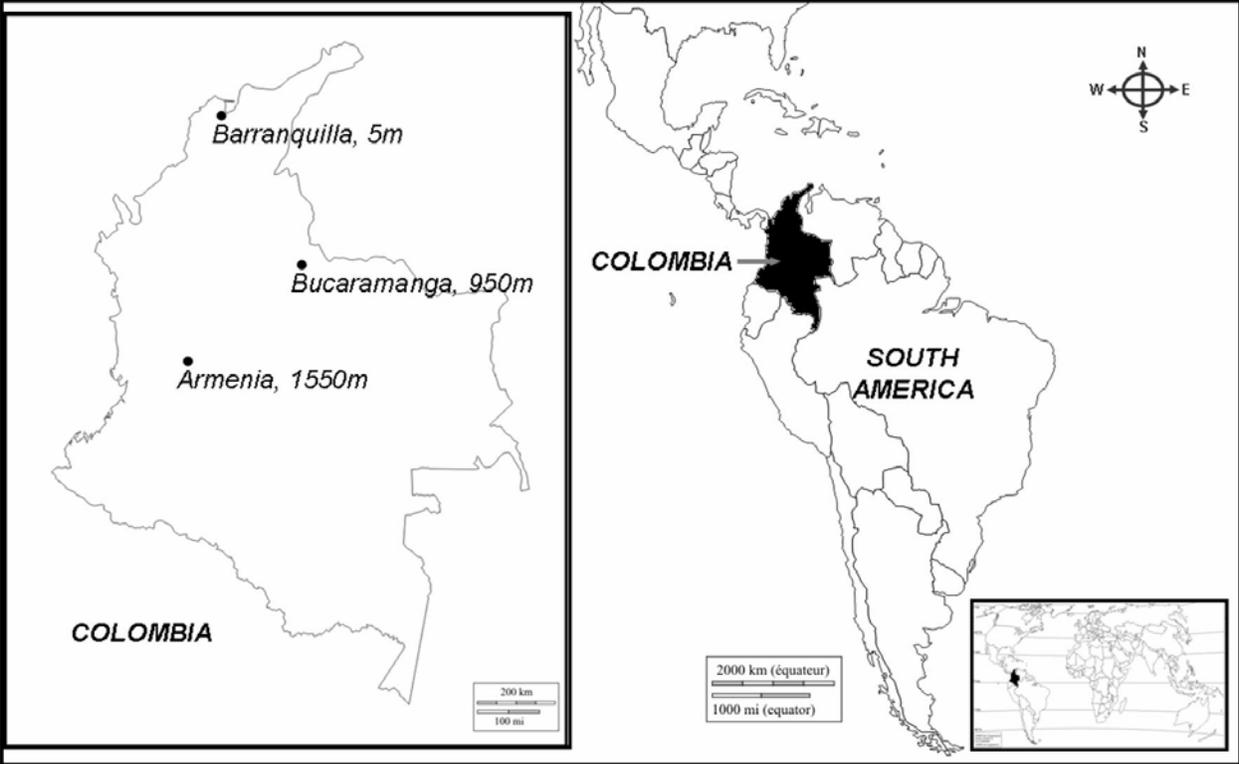


Figure 2-1. Study cities within Colombia.

A



B



Figure 2-2. Water storage vessels in study areas. A) Typical lavaderos of Armenia (left) and Bucaramanga (right). B) Diverse water storage vessels of Barranquilla.

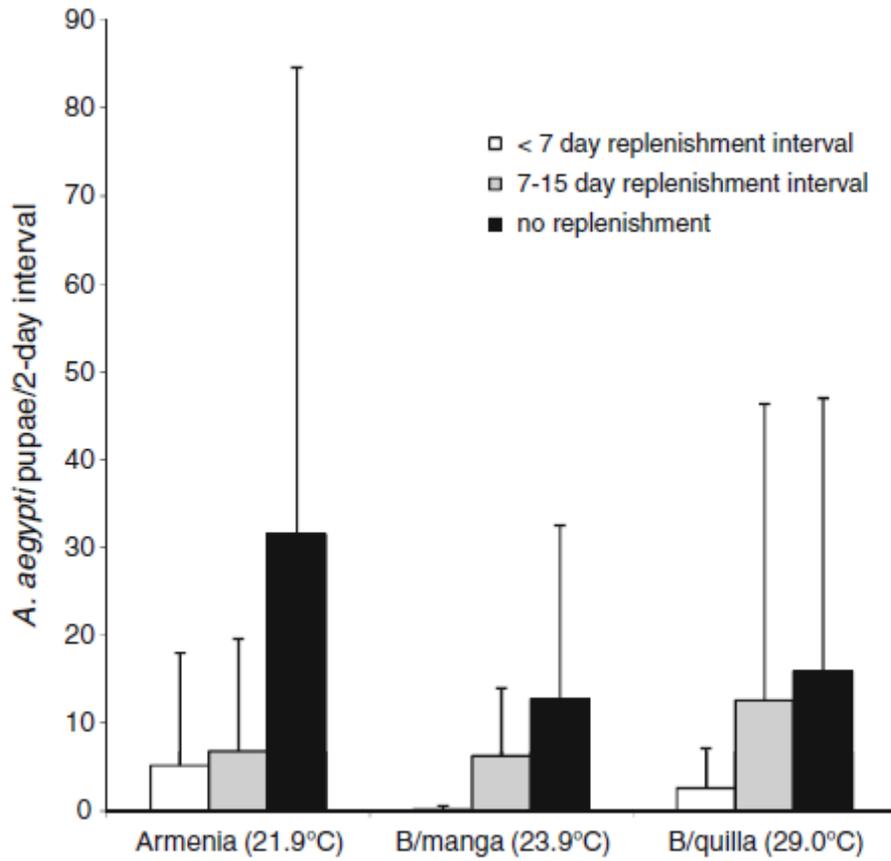


Figure 2-3. Effect of emptying interval on the rate of *A. aegypti* pupal production (mean, standard error) in larval infested vessels in three dengue endemic cities with different temperatures.

CHAPTER 3  
CELL SIZE AND NUMBER RELATIONSHIPS IN *Aedes aegypti* (L.) WINGS SHOW  
DIFFERENTIAL AND INTERACTIVE EFFECTS OF TEMPERATURE AND FEEDING  
RATE ON MOSQUITO MORPHOLOGY

**3.1 Introduction**

Temperature and food availability in the habitats of developing ectotherms are fundamental determinants of variation in adult size. In the mosquito *Aedes aegypti* (L.), ecological processes affecting adult size may also affect variation in the capacity of populations to transmit diseases such as dengue and chikungunya. This is because adult size has been correlated with maturation rate (Rueda et al., 1990; Tun-Lin et al., 2000), fecundity (Steinwascher, 1982; Nakasathit and Scott, 1998; Ponlawat and Harrington, 2007), biting habits (Klowden et al., 1988; Nasci, 1991), dispersal (Maciel de Freitas et al., 2007), survival (Reiskind and Lounibos, 2009), and infectiousness (Alto et al., 2008; Westbrook et al., 2009). In these studies size variation was experimentally generated across gradients of resource limitation or temperature; however in adult mosquitoes collected in field settings there is currently no way of distinguishing between these two effects on size.

Thermal and nutritional constraints affect attainment of two developmental milestones in mosquitoes: critical weight (minimum weight required for pupation) and asymptotic or final weight, both of which are lower in males than in females. The interval to cessation of growth (ICG) is the period between attainment of the critical and final weights, during which up to 50% of total growth may occur under optimal feeding conditions (Davidowitz and Nijhout, 2004, Nishiura et al., 2007; Telang et al., 2007). Increasing temperature [within thermal optima] reduces both critical and final weights in *A. aegypti* (Chambers and Klowden, 1990; Rueda et al., 1990); this generates a crossing of growth trajectories such that larvae reared in warm conditions reach the critical weight sooner than larvae in cooler conditions, but the latter eventually reach a

larger asymptotic size. In contrast, food variation generates nested growth trajectories such that larvae reared with more food reach the critical weight faster and grow to a larger final weight. Thus, variation in food intake and temperature induce diverging growth trajectories specifically during the ICG. Because not all body components grow at the same rate over the course of development it may be that the differential impacts of food and temperature on growth trajectory in mosquitoes may be observed in morphologic indicators of allometry.

Body size in multicellular organisms can be viewed as having two components: the size of cells and their number. In insects, body size is determined by the size of the surface epidermis that secretes the exoskeleton; thus, epidermal cells are the key building block of overall size (French et al., 1998). In Dipterans wing cell size is conveniently estimated based on the density of trichomes (wing hairs), each of which represents a single cell. Arendt (2007) reviewed a total of 14 studies comparing cell size and number relationships in *Drosophila melanogaster*, all of which analyzed size and number of epidermal wing cells. In the only study that we found comparing temperature effects on cell size in multiple organs, Azevedo et al. (2002) found that temperature affected epidermal cell sizes consistently in fruit fly wings, feet and eyes. In general, studies on *D. melanogaster* indicate that colder temperature affects female size through cell size rather than cell number, whereas in males colder temperature increases both cell size and number. (Azevedo et al., 2002; French et al., 2002; de Moed et al, 1997; Arendt, 2007). To our knowledge no studies have looked at the effects of cell size and number on the function or shape of wings. In the only study to investigate the combined effects of temperature and food in *D. melanogaster*, de Moed et al. (1997) found differential and interactive effects of temperature and food on cell size and number. Because organisms in natural environments simultaneously experience resource and temperature variation there is a need for more research on their

combined effects on allometric growth (Arendt, 2007). While the de Moed et al. (1997) study suggests that cell size and number relationships may reflect the mechanistically diverging impacts of food and temperature on development, there is no reason to expect the relationships in observed in *D. melanogaster* to hold true in mosquitoes.

In *A. aegypti*, up to 80% of growth may occur in the fourth instar (Telang et al., 2007), including the thoracic proliferation of imaginal discs, which give rise to the wing epidermis (Christophers, 1960; Nishiura, 2002). Therefore, wing morphology is likely to be highly sensitive to the environmental conditions that contribute to size variation in field collected mosquitoes. *A. aegypti* winglength has been shown to increase with lower temperature (Tun-Lin et al, 2000) and heightened resources, induced either by low larval densities or high food input (Bedhomme et al, 2003; Jirakanjanakit et al., 2007). Bedhomme et al (2003) found that heightened feeding time increased the winglength of female *A. aegypti* more so than males. Jirakanjanakit et al (2007) also found significant allometric effects of food and density treatments on the geometry of wing veins; moreover, this study showed that allometric effects were similar across sexes in mid-range food/density treatments but not at extremely high and low density conditions. While variation in both resource and temperature conditions has been shown in field *A. aegypti* habitats (Arrivillega and Barrera, 1996; Strickman et al., 2003; Barrera et al., 2006b; Tun-Lin et al., 2000), no studies to our knowledge have investigated their differential or interactive effects on adult wing morphology.

Here, we investigated cell size and cell number in wings of *A. aegypti* reared under temperature and food conditions designed to simulate those encountered in dengue endemic areas of different altitudes in Colombia. In particular, we determined the independent and interactive effects of food and temperature treatments on differences between standardized

indices of cell size and number, highlighting their contribution to overall wing size for both males and females.

## **3.2 Materials and Methods**

### **3.2.1 Experimental Rearing of *Aedes aegypti***

This study was carried out in Universidad Nacional de Colombia, Bogotá, Colombia (elevation: 2640m, latitude 4°N, mean temp: 13.0°C), in rearing conditions designed to simulate typical domestic habitats at different dengue-endemic elevations in Colombia. Twenty newly hatched *A. aegypti* larvae (F2) from Barranquilla, Colombia (altitude 5m, latitude 10°N, mean temperature 26-29°C), were raised on different levels of standardized household detritus (see below) in 20 L buckets (filled with at least 19.5 L of water), each with a 5W aquarium heater (Aquarios S.A., Bogotá, Colombia) submerged at the bottom. Buckets were placed in one of two temperature treatments: cold buckets were exposed to Bogotá indoor temperatures, and warm buckets were placed in a non-insulated room (3x4m) maintained at 25°C by an electric space heater. These treatments were standardized prior to experiments so that the cold group water would fluctuate between 21-23°C and the warm group between 27-29°C. Water temperatures were measured hourly in 1 bucket within each temperature group, using an I-button temperature logger (Embedded Data Systems, Lawrenceburg, KY) submerged at the bottom of each vessel and daily in each bucket (because of only a few loggers available).

In order to prepare a standardized household detritus for food treatments, we distributed plastic bags in 30 homes located in a dengue endemic Colombian city and asked residents to sweep the area immediately surrounding their water storage vessel and deposit 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 0.25mm<sup>2</sup>. 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 vessel included both layers and was measured on a balance with approximately ±5mg error.

In order to simulate a range of food conditions that *A. aegypti* may experience in household vessels, two consecutive experiments were carried out in each temperature treatment. In Experiment 1, 5 groups of 3 replicate vessels received one of the following treatments every 3 days beginning on the day when the 20 newly hatched larvae were added (day 0): 50, 100, 200, 400 and 800 mg of sediment (hereby referred to by the respective daily application rates: 16.7, 33.3, 66.7, 133.3, 266.7 mg/day) Based on the pupation results of Experiment 1 (hereby called high food experiment), we sought to increase food scarcity in Experiment 2 (hereby called low food experiment). However we observed that 40 mg was the minimum amount of food applied that could contain matter from both of the layers described above. Accordingly, in the low food experiment, we varied food input frequency instead of amount. Groups of 4 replicate vessels per temperature were each assigned one of the following daily probabilities of addition of 40 g of sediment: 0.1, 0.25, 0.5, 0.75. A random number generator determined the days in which each replicate received a food treatment. We used this method instead of food application at constant intervals because constant food input under food limiting conditions may stall development in the L4 stage (Gilpin and McClelland, 1979). Food application rates (± s.e.) for each group were

the following: 2.9 ( $\pm 10.5$ ), 11.4 ( $\pm 18.4$ ), 20.0 ( $\pm 20.4$ ) and 27.8 ( $\pm 18.8$ ) mg/day. Because these rates are calculated over the time of the last pupation event and are not necessarily indicative of the food experienced by most of the resulting mosquitoes, we hereby use the following expected values to define food application rates in the low food experiment: 4, 10, 20 and 30 mg/day. Experiments 1 and 2 gave a total of 62 vessels. Fourth instar larvae (L4) and pupae were counted daily until all larvae died or pupated. All pupae were removed and allowed to complete development at 25°C, as we are unaware of evidence linking temperature in the pupal stage with wing development.

### **3.2.2 Wing Photography and Image Analysis**

The right wing of each surviving mosquito with undamaged wings was mounted on a cover slip, with the dorsal side facing up, using a 2:1 mixture of water with transparent paper glue. Digital cameras and Optika Vision Pro software were employed in order to produce two images of each wing: 1) a 2048 x 1536 pixel image of the entire wing using a dissecting microscope (2.5x) (Figure 3-1A) and 2) a 480 x 640 pixel image of a 0.0108 mm<sup>2</sup> area in the third posterior wing cell between the 1<sup>st</sup> and 2<sup>nd</sup> anal veins using a compound microscope (40x) (Figure 3-1B).

Image analyses were conducted using Image J software (National Institutes of Health). The software automatically calculated the area enclosed by a trace of the perimeter of the wing using the image in Figure 3-1A. It also performed an automated hair count by dichotomizing the color spectrum in Figure 3-1B and counting the number of black points. Because wing veins, split hairs, or discontinuities in the background color due to mounting imperfections were all potential sources of error, counts were conducted on a smaller 0.0032 mm<sup>2</sup> square area *within* Figure 3-1B. For each image we located the square that maximized the visualization of hairs and

minimized blurriness and debris. Images with noticeable errors in dichotomization of hairs were discarded. In the absence of evidence on heterogeneities in epidermal cell size across mosquito wings, we considered that our procedure achieved an appropriate balance between including a representative number of hairs in each take (~45-85) and maximizing image quality. Mean cell size was estimated by the reciprocal of hair counts, and an index of wing cell number was defined as wing area divided by the estimate of mean cell size.

### 3.2.3 Data Analysis

Due to the variation in food input schedules and contrasting developmental strategies across sexes, data were analyzed separately for males and females and in the high and low food experiments. We first determined the effects of food input rate, temperature treatment and the food-temperature interaction on the two variables measured independently for each observation, wing size and cell size. Subsequently, we analyzed whether treatments had a larger impact on cell size or number.

Because of the large difference in scaling of wing and cell size, we used Z-scores to standardize wing area ( $Z_{wing}$ ), mean cell size ( $Z_{size}$ ) and cell number index ( $Z_{number}$ ). Z-scores are defined as  $Z=(x_i - \bar{x})/\sigma$ , where  $x_i$  is the measured value for each mosquito  $i$ ,  $\bar{x}$  is the mean and  $\sigma$  is the standard deviation of each of the three measurements outcomes. Z measures the magnitude and direction of the deviation of each observation from the overall mean, positive if above the mean, negative if below, generating a standard normal distribution in the overall data with mean of 0 and standard deviation of 1. We determined whether treatments had a larger impact on cell size or cell number through the difference in z-scores, defined for each mosquito  $i$  as:

$$Z_{diff,i} = Z_{size,i} - Z_{number,i} \quad (3-1)$$

According to this definition, if a particular treatment causes a disproportionate increase in cell size compared to cell number, then  $Z_{\text{size},i}$  will be large compared to  $Z_{\text{number},i}$ , and  $Z_{\text{diff},i}$  will be a large positive number. In the opposite case, that a treatment results in an inordinate increase in cell number compared to cell size,  $Z_{\text{diff},i}$  will be negative. Therefore, predictors positively associated with  $Z_{\text{diff}}$  will have a larger impact on cell size and a negative association indicates a larger impact on cell number. No association with  $Z_{\text{diff}}$  means no allometric effect.

Because mosquitoes were produced from 62 different buckets, each exposed to random differences in our heterogeneous field-collected food, in addition to a highly unbalanced design with many containers yielding only 1 or 2 individuals per sex used in wing analyses, we used multiple random-effects, maximum likelihood (ML) regression using the procedure *xtreg* in STATA 8.0 Statistical Software (StataCorp., College Station, TX). Container was the group variable assigned random effects. We quantified the combined effects of temperature (warm group = 27-28°C versus cool group = 21-22°C), the Z-score of feeding rate (hereby referred to as food), and the food temperature interaction (hereby referred to as warm\*food) on the dependent variables  $Z_{\text{wing}}$ ,  $Z_{\text{size}}$  and  $Z_{\text{diff}}$ . Wald tests ( $\alpha = 0.05$ ) were employed to determine the significance of regression coefficients. A separate model was constructed for each sex and food input experiment for a total of four models for each dependent variable. By evaluating significant associations with  $Z_{\text{wing}}$ ,  $Z_{\text{cell}}$  and  $Z_{\text{diff}}$  conclusions were drawn as to whether treatments effects on overall wing size were due to a larger effect on cell size or number.

### 3.3 Results

Mean daily temperatures across containers were 22.2°C (range across vessels: 21.7-22.5) and 28.2°C (range across vessels 27.9-28.6) in cool and warm treatments, respectively, which were underestimates, as temperature in each container was generally measured in the cooler

morning hours. In the containers that were monitored hourly, average daily minimum and maximum temperature over the two experimental trials were 22.0 and 22.9°C (mean 22.5°C) in the cold treatment and 27.9 and 29.1°C (mean 28.5°C) in the warm treatment.

A total of 128 and 216 mosquitoes were analyzed in the low and high food experiments, respectively, as compared to 231 and 312 pupae that were harvested in each. This discrepancy was due to a combination of relatively high pupal mortality in food limited treatments, newly emerged mosquitoes that drowned or whose right wing was otherwise folded or damaged, and inadequate wing mounting. Mean time to pupation decreased with increasing food input and was lower in the warm group in all feeding regimes (Figure 3-2). Time to pupation in the highest food treatments in the low food experiment was higher than that of the lowest feeding groups of the high food experiment (Figure 3-2). Given that mean food addition rate was comparable among these groups, the data suggest that the random feeding method may have had an effect on lowering development rate.

Total wing area of females was significantly larger in those mosquitoes reared in the cold temperature as opposed to the warm in both experiments (Table 3-1, Figure 3-3). Increasing food addition rate was associated with larger female wing area in the high food experiment (Figure 3-3B) but not in the low food experiment (Figure 3-3, Table 3-2). The lower temperature treatment was also significantly associated with larger cell size in both experiments (Table 3-2) although the effects were less pronounced than for wing size (Figure 3-3). In the high food experiment increased feeding rate was not associated with cell size. In the low food experiment increased feeding rate was associated with larger epidermal cells in the warm treatment but not in the cold (Figure 3-3), as evident in the significant warm\*food interaction term in the model of female cell size for this experiment (Table 3-1). In the two lowest food groups (4 and 10 mg/day in Figure 3-

3) cell size was noticeably larger in the cold treatment; this trend switched at 30mg/day, with increasing mean cell size in the warm treatment (Figure 3-3).

As with females, the lower temperature treatment significantly increased male wing size in both experiments, whereas increased food addition was associated with larger wings only in the high food experiment and not associated with cell size in either experiment (Figure 3-4, Table 3-2). Unlike females the cold treatment increased cell size only in the low food experiment. In the high food experiment, there was a significant food\*warm interactive effect, such that in low food conditions males reared in the cold treatment had larger cells, but in the higher feeding groups mean cell size was larger in the warm treatment (Table 3-2, Figure 3-4).

There was a significant warm\*food interactive effect on  $Z_{diff}$  in females in the low food experiment, indicating that the relative effects of food on cell number and size differed between temperature treatments (Table 3-3). In particular, the positive coefficient indicates that in the warm group increasing food input rate is associated with a larger increase in cell size than in cell number whereas in the cold group higher food induces a larger increase in cell number. This is evident in the crossing of the regression lines in Figure 3-5a with mosquitoes below the  $y=0$  plane having a larger cell number relative to cell size. Neither of the treatments or their interaction are significantly associated with  $Z_{diff}$  in males in the low food experiment or females in the high food experiment (Table 3-3). In males in the high food experiment, food input was associated with a larger increase in cell number than in cell size, as is evidenced by the larger density of dots (mosquitoes) above the  $y=0$  plane in the low food input rates as compared to the high food input rates (Figure 3-5b); however, this effect varied among temperature groups as evidenced in the significant, positive warm\*food interaction (Table 3-3). The larger impact of

food on cell number relative to cell size was more pronounced in the cold treatment (Figure 3-5b).

Table 3-4 qualitatively summarizes the significant model results for wing size, cell size and  $Z_{diff}$ , indicating whether treatment effects on overall size were brought about by a larger effect on cell size or cell number. Mosquitoes reared in the cold treatment had larger wing and cell size (i.e. a significantly negative effect of warm), with the exception of males in the high food experiment (Table 3-4). However, the lack of a significant association with  $Z_{diff}$  indicates that the effects of the cold treatment on wing size were not generated through a significantly greater effect on cell size relative to cell number or vice versa. Food addition rate increased wing size in both sexes only in the high food experiment. In males this was due to a larger increase in cell number than in cell size (significant negative association with  $Z_{diff}$ ), whereas no allometric effect was detected in females. Interestingly, the warm\*food interaction term was not associated with wing size but did have a positive effect on cell size and  $Z_{diff}$  for males in the high food experiment and females in the low food experiment. Given that the cold treatment, but not food, increased female wingsize and cell size in the low food experiment, the warm\*food interactive effect on  $Z_{diff}$  suggests that the cold treatment increased wing size through a larger increase in cell size at low feeding rates but through cell number as high feeding rates. For males in the high food experiment, both food and temperature increased wing size, but neither had a significant effect on cell size (Table 3-2, Figure 3-4). Thus the interactive effect on  $Z_{diff}$  mean that the relative contribution of cell size and number to the observed wing size variation was specific to both temperature group and food application rate (Figure 3-5)

### **3.4 Discussion**

Although both thermal and resource regulation in aquatic stages are key processes shaping the size, abundance and distribution of mosquitoes, surprisingly little is known about the

underlying mechanisms through which they affect growth and development. Here, for the first time that we know of in a mosquito, we demonstrate that independent, interactive and sexually dimorphic impacts of food and temperature in the larval environment can be observed in adult morphology. We show that while both lower temperature and heightened feeding increase wing size, they differ in the magnitude of their impacts on epidermal cell size and cell number in *A. aegypti*, reared under conditions simulating those observed in dengue endemic areas in Colombia. Moreover, our results indicate that temperature and resource availability have interactive effects on mosquito development, such that the effect of each changes with the level of the other.

Numerous prior studies show that wingsize in *A. aegypti* increases with lower rearing temperatures and increased food provision (Rueda et al, 1990; Tun-Lin et al, 2000; Jirakanjanakit et al., 2007), but to our knowledge no studies have investigated food and temperature effects on cell size. In *D. melanogaster*, the taxonomically closest organism on which this data exists, studies consistently show that lower temperature increases cell size, with lesser or no impact on cell number (Arendt, 2007). In addition, DeMoed et al (1997) found that increased food has larger effects on *D. melanogaster* cell number than on cell size. In the experiments presented here *A. aegypti* raised at 22°C had both larger wings and larger epidermal cells than those raised at 28°C. However, our data indicate that the effect of the cold treatment on cell size was approximately similar to its effects on cell number, as evidenced in the consistently non-significant impact on  $Z_{diff}$  in both experiments and sexes (Tables 3-3 and 3-4). Food application increased wing size only in the high food experiment but was not associated with cell size in either sex. Moreover, in males, increased food had a significantly larger impact on cell number than on cell size, particularly in the cold treatment (Table 3-3, Figure 3-5), in accordance

with DeMoed et al (1997). These results demonstrate morphologically distinct effects of food and temperature and suggest that they differ in their impacts on cell growth and division.

Also consistent with DeMoed et al (1997) we found significant interactive effects of food and temperature on cell size, such that heightened food input increased cell size in the warmer group, but not the colder group. This interactive effect caused a positive association with  $Z_{diff}$ , indicating that food had a larger effect on cell size than cell number in the warm treatment, but had a larger effect on cell number in the cold treatment. For females, this occurred in the low food experiment and for males it occurred in the high food experiment. However, unlike *D. melanogaster* (DeMoed et al, 1997), this interaction was not present in overall wing size. The cold treatment, but not feeding rate, increased wing size in females in the low food experiment. We suggest that in extremely food limited habitats (4 and 10 mg/day in our experimental conditions), lower temperature increases female size through larger increases in cell size than cell number, whereas at higher food levels both cell number and cell size contribute. In males in the high food experiment, both increased food and lower temperature associated with larger wings, whereas neither had an independent effect on cell size (Table 3-2, Figure 3-4). Therefore, the warm\*food interaction indicates that the relative contribution of cell size and number to environmental variation in male wing size is specific to the combination of temperature and resource conditions (Table 3-4).

Morphologically, the size of insects is the product of the number and size of epidermal cells composing the exoskeleton, whereas physiologically, size is a product of the growth rate and the duration of growing stages (Davidowitz and Nijhout, 2004). In mosquitoes food resources increase growth rate; by contrast lower temperature prolongs growing time while increased temperature promotes increased food assimilation rate (Rashed and Mulla, 1989). Sex-specific

development strategies have also been shown to interact with environmental conditions. For example increased resources have been shown to favor heightened energy storage and decreased time to maturation in male *A. aegypti*, whereas in females increased resources have greater effects on structural growth and size and smaller effects on development time (Timmermann and Biegel, 1999; Bedhomme et al, 2003). We can speculate as to how these sexually dimorphic ecological relationships fit with the results presented here. For example, females in the cold treatment in the 4 and 10mg/day groups (low food experiment) would have had exceptionally low food uptake and energy storage; thus cold temperature may have caused a disproportionate increase in cell size over number as a consequence of the need to maximize size by prolonging development. By contrast males in the warm treatment in the 133.3 and 266.7 mg/day groups (high food experiment) would have had exceptionally high food assimilation rates; therefore a disproportionate increase in cell size in the wing epidermis may reflect heightened cellular energy storage [in other tissues] within a very short window of development. Assessing the validity of these assertions, however, would require understanding how the Barranquilla *A. aegypti* strain used in our experiments may have influenced the results.

In field surveys of *A. aegypti* populations throughout the world larval nutrient indicators consistently demonstrate large variation across urban *A. aegypti* habitats (Subra and Mouchet, 1984; Tun-Lin et al., 2000; Strickman et al., 2003; Arrivillaga and Barrera, 2004; Barrera et al., 2006b). In a study of geometric relationships in *A. aegypti* wing veins, the allometric effects of resource availability on wing geometry changed at extremely low and high larval densities/food inputs, and these treatments were discarded in the statistical analysis (Jirakanjanakit et al., 2007). However, given that food limitation in larval stages is considered a critical process in determining the dynamics of adult *A. aegypti* populations (Southwood et al., 1972),

understanding the effects of “extreme” resource conditions may be highly relevant for field *A. aegypti*. Moreover, as heterogeneity in the food particles available to *A. aegypti* affects larval nutrition (Rashed and Mulla, 1989), heterogeneous detritus and microorganisms that larvae encounter in field habitats are likely to bear little resemblance to standardized laboratory diets. Thus, we chose an experimental design which maximized the similarity of our experiments with the wide range of resource conditions experienced by *A. aegypti* in the field, and minimized the potentially large stochastic effects of administering small quantities of heterogeneous detritus food. The use of random food input in the low food experiment introduced a further source of variance, which combined with the variation in frequency instead of quantity of food input, made it impossible to combine the analysis in order to better isolate the independent effects of food abundance. Nonetheless, the design employed was able to generate extremely nutritionally stressed mosquitoes as evidenced in the mean development times on the order of 20-25 days (males and females combined) (Figure 3-2). Despite small sample size in the female low food experiment, the data indicates that the wide range of food abundance was essential in detecting interactive effects of heightened feeding rate and temperature. Although alternative designs of food application and the usage of replicate incubators for temperature treatments may have generated clearer results, the resemblance of our experiment to field conditions may increase the prospects for field validation.

The food-temperature interactions that we demonstrate in both sexes may represent a first step in developing the means to assess larval habitat quality based on adult characteristics in this important disease vector. As a first approximation to field testing our results, adult *A. aegypti* could be collected across a range of climates and habitats. We predict that as nutritional stress experienced in habitats decreases, the difference between standardized indices of cell size and

number should increase at 28°C and decrease at 22°C. Furthermore, variation in indices should occur at the low end of the resource spectrum in females and at the high end in males.

Considering the limitations of our experimental design, field validation of these predictions could represent a significant advance in understanding the regulatory processes in natural *A. aegypti* populations. Moreover, further experimental studies that allow comparison of our findings to other arthropod vectors and *A. aegypti* strains from different climates can provide insights on how natural selection in the face of warming climates may impact vector borne disease.

Table 3-1. Random effects models of wing area and mean cell size in female *A. aegypti* in high and low food experiments

| Dependent variable              | Treatment | High food experiment<br>n=104 |       | Low food experiment<br>n=43 |       |
|---------------------------------|-----------|-------------------------------|-------|-----------------------------|-------|
|                                 |           | Coefficient<br>(95% CI)       | P     | Coefficient<br>(95% CI)     | P     |
| Wing area<br>(z-score)          | Warm      | -1.27<br>(-1.56, -0.99)       | <0.01 | -1.40<br>(-1.85, -0.95)     | <0.01 |
|                                 | Food      | 0.50<br>(0.25, 0.75)          | <0.01 | -0.07<br>(-0.25, 0.39)      | 0.68  |
|                                 | Food*warm | -0.21<br>(-0.52, 0.09)        | 0.18  | -0.15<br>(-0.60, 0.31)      | 0.53  |
| Mean cell<br>size (z-<br>score) | Warm      | -0.46<br>(-0.81, -0.11)       | 0.01  | -0.62<br>(-1.16, -0.07)     | 0.03  |
|                                 | Food      | 0.15<br>(-0.17, 0.48)         | 0.35  | -0.29<br>(-0.69, 0.10)      | 0.14  |
|                                 | Food*warm | -0.07<br>(-0.46, 0.32)        | 0.74  | 0.65<br>(0.10, 1.21)        | 0.02  |

Note:

\*Using *xtreg* procedure in STATA 8.0.

Table 3-2. Random effects models of wing area and mean cell size in male *A. aegypti* in high and low food experiments

| Dependent variable              | Treatment | High food experiment<br>n=112 |       | Low food experiment<br>n=85 |       |
|---------------------------------|-----------|-------------------------------|-------|-----------------------------|-------|
|                                 |           | coefficient<br>(95% CI)       | P     | coefficient<br>(95% CI)     | P     |
| Wing area<br>(z-score)          | Warm      | -1.30<br>(-1.60, -1.01)       | <0.01 | -1.33<br>(-1.73, -0.93)     | <0.01 |
|                                 | Food      | 0.38<br>(0.19, 0.57)          | <0.01 | 0.15<br>(-0.14, 0.44)       | 0.31  |
|                                 | Food*warm | -0.15<br>(-0.40, 0.11)        | 0.27  | -0.14<br>(-0.55, 0.27)      | 0.50  |
| Mean cell<br>size (z-<br>score) | Warm      | -0.31<br>(-0.71, 0.10)        | 0.14  | -0.66<br>(-0.99, -0.34)     | <0.01 |
|                                 | Food      | -0.27<br>(-0.55, 0.01)        | 0.06  | -0.03<br>(-0.22, 0.29)      | 0.79  |
|                                 | Food*warm | 0.54<br>(0.14, 0.94)          | 0.01  | -0.04<br>(-0.37, 0.33)      | 0.83  |

Note:

\*Using *xtreg* procedure in STATA 8.0.

Table 3-3. Random effects models of  $Z_{diff}$  in female and male *A. aegypti* in high and low food experiments

| Sex    | Treatment | High food experiment    |       | Low food experiment     |      |
|--------|-----------|-------------------------|-------|-------------------------|------|
|        |           | coefficient<br>(95% CI) | p     | coefficient<br>(95% CI) | p    |
| Female | Warm      | 0.53<br>(-0.07, 1.12)   | 0.08  | 0.51<br>(-0.45, 1.47)   | 0.30 |
|        | Food      | -0.31<br>(-0.86, 0.24)  | 0.27  | -0.55<br>(-1.23, 0.14)  | 0.12 |
|        | Food*warm | 0.14<br>(-0.51, 0.79)   | 0.67  | 1.26<br>(0.3, 2.23)     | 0.01 |
| Male   | Warm      | 0.58<br>(-0.16, 1.32)   | 0.13  | 0.12<br>(-0.46, 0.71)   | 0.68 |
|        | Food      | -0.83<br>(-1.33, -0.33) | <0.01 | -0.05<br>(-0.51, 0.41)  | 0.83 |
|        | Food*warm | 1.08<br>(0.38, 1.78)    | <0.01 | 0.08<br>(-0.52, 0.67)   | 0.80 |

\*Using *xtreg* procedure in STATA 8.0.

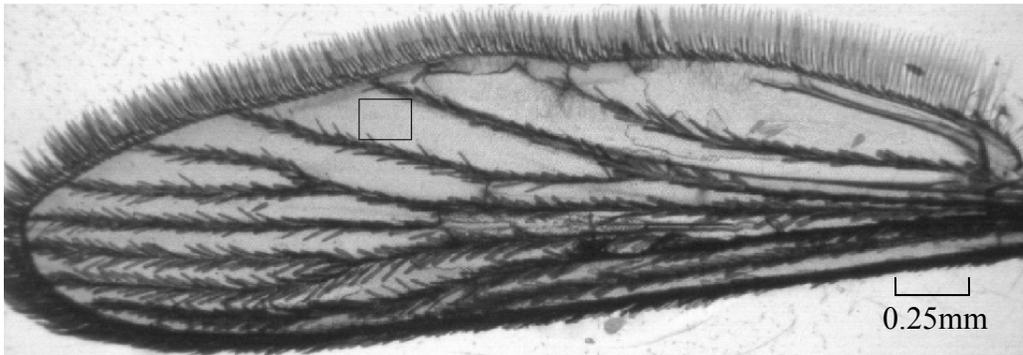
Table 3-4. Summary table of significance of main and interactive effects of food and temperature treatments in random effects model of wing size, cell size and  $Z_{diff}$

| Sex     | Treatment | High food experiment |            |            | Low food experiment |            |            |
|---------|-----------|----------------------|------------|------------|---------------------|------------|------------|
|         |           | $Z_{wing}$           | $Z_{cell}$ | $Z_{diff}$ | $Z_{wing}$          | $Z_{cell}$ | $Z_{diff}$ |
| Males   | Warm      | -                    | NS         | NS         | -                   | -          | NS         |
|         | Food      | +                    | NS         | -          | NS                  | NS         | NS         |
|         | Warm*Food | NS                   | +          | +          | NS                  | NS         | NS         |
| Females | Warm      | -                    | -          | NS         | -                   | -          | NS         |
|         | Food      | +                    | NS         | NS         | NS                  | NS         | NS         |
|         | Warm*Food | NS                   | NS         | NS         | NS                  | +          | +          |

Note:

NS means non-significant at  $\alpha=0.05$

A



B

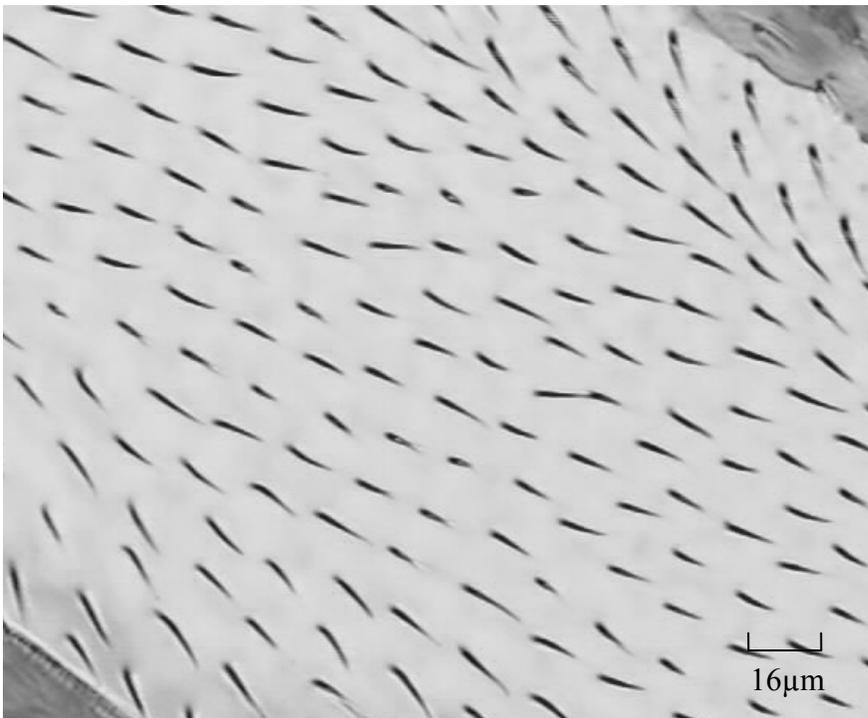
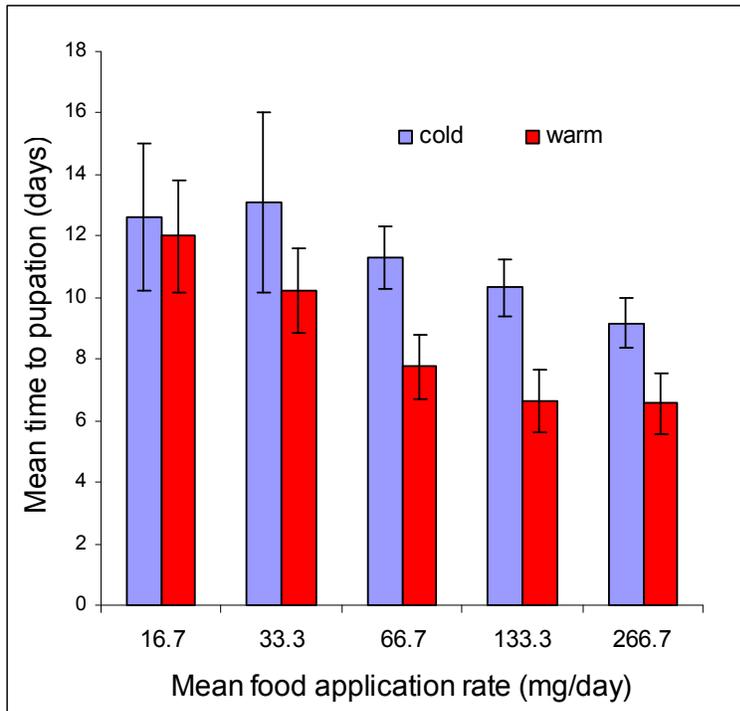


Figure 3-1. Images used to determine wing size and epidermal cells size in *A. Aegypti*. A) Image of *A. aegypti* wing used to measure wing area. Square indicates region in which hair counts were conducted. B) Image in 3<sup>rd</sup> posterior wing cell used count hairs in order to estimate cell size.

A



B

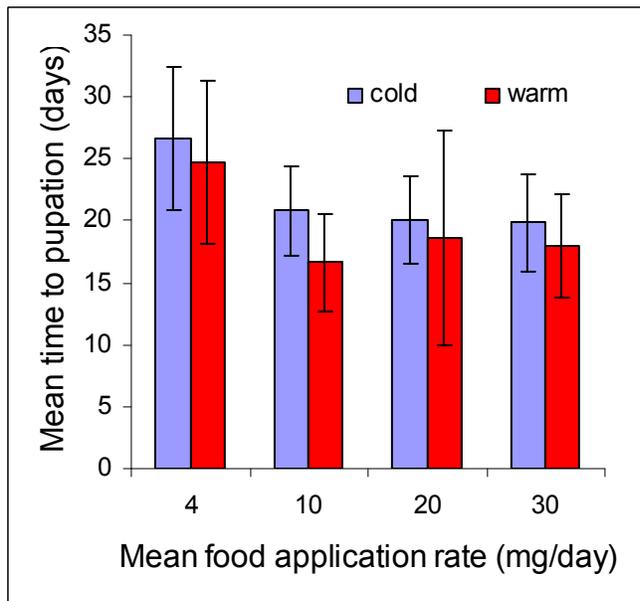


Figure 3-2. Mean time to pupation among experimental treatments of food and temperature. A) High food experiment (n=312). B) Low food experiment (n=231). Figure comprises both males and females and all pupae excluded from the wing analyses due to mortality, damaged wings, or inadequate mounting.

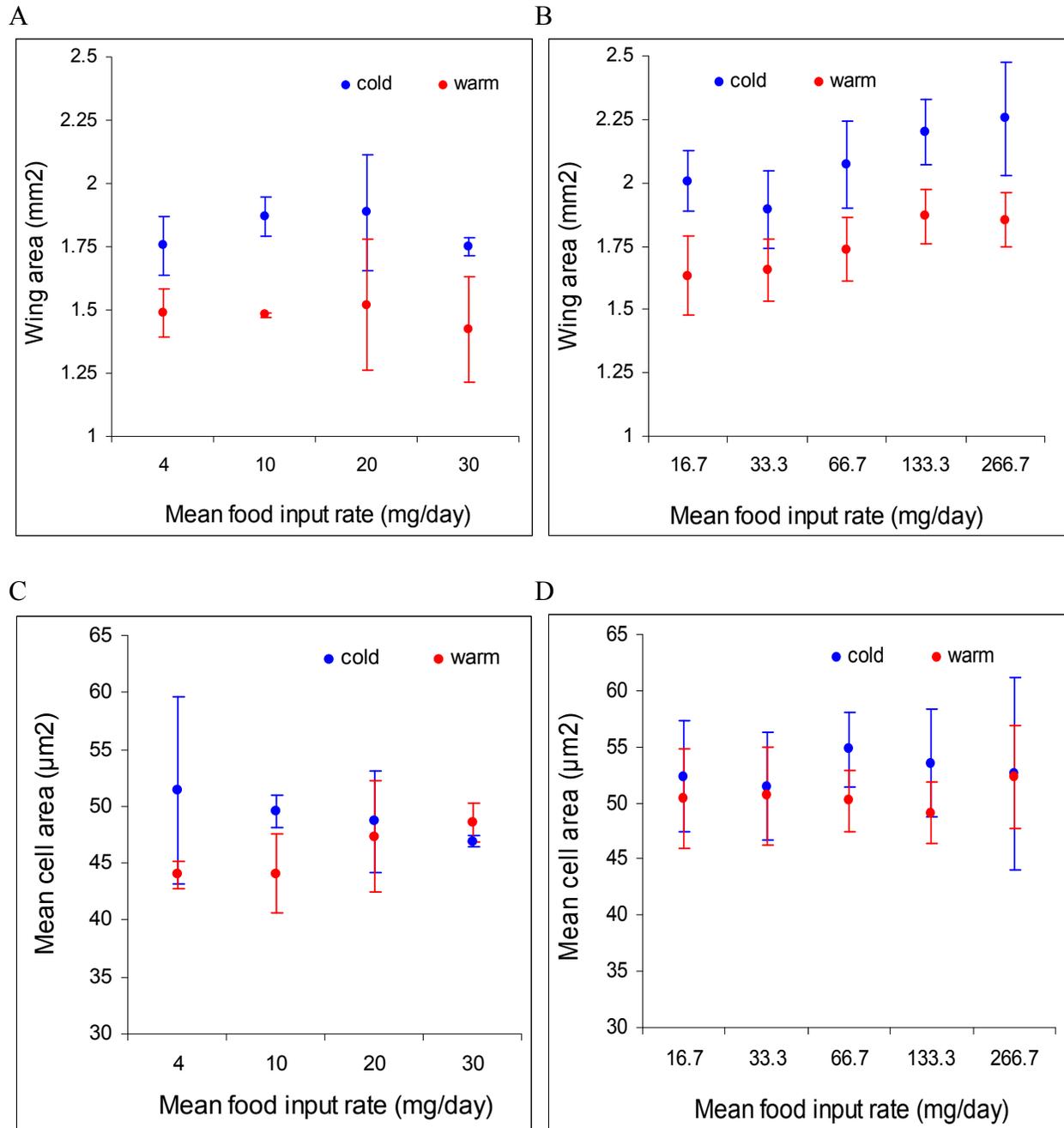


Figure 3-3. Wing measurements in female *A. aegypti* across food and temperature treatments. A) Wing area low food experiment. B) Wing area high food experiment. C) Average epidermal cell area in low food experiment. D) Average epidermal cell area in high food experiment.

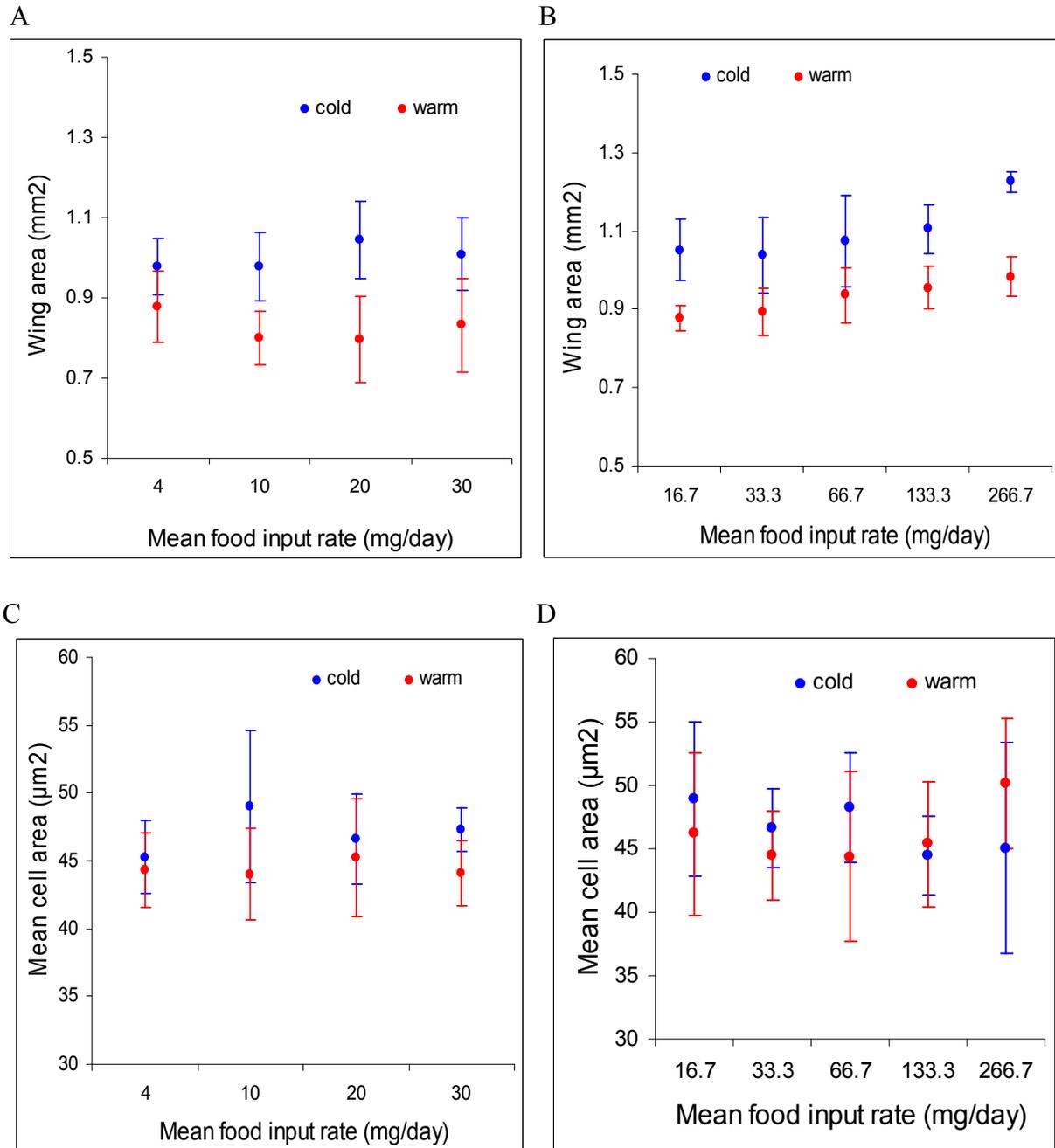
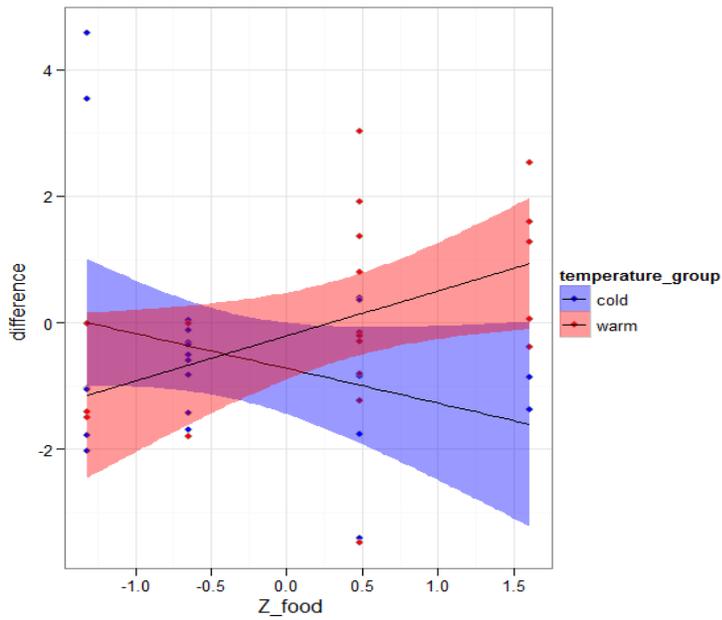


Figure 3-4. Wing measurements in male *A. aegypti* across food and temperature treatments. A) Wing area low food experiment. B) Average epidermal cell area low food experiment. C) Wing area high food experiment. D) Average epidermal cell area high food experiment.

A



B

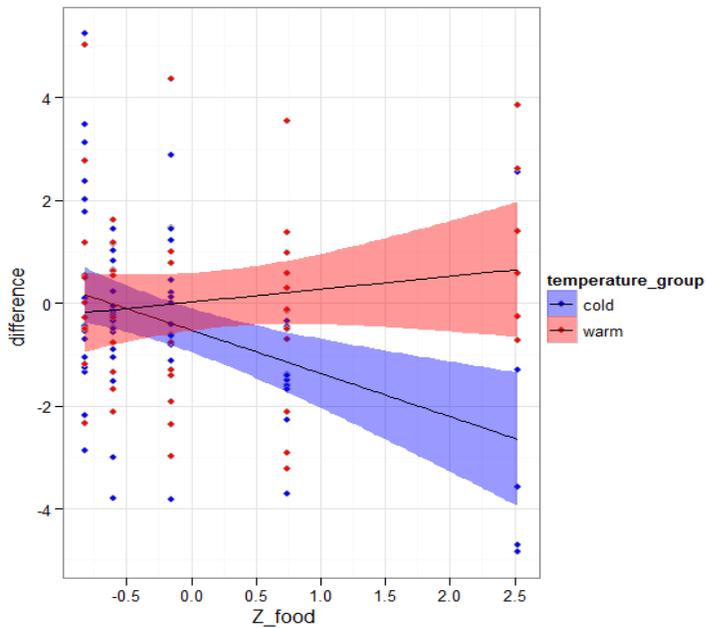


Figure 3-5. Significant interactive effects of food and temperature on  $z_{\text{diff}}$  (labeled as difference in y-axis). A) Females in low food experiment. B) Males in high food experiment. Black lines are the linear regression of feeding rate in each temperature. Dots are individual mosquitoes; black lines depict regression of food effects within each temperature group.  $Z_{\text{food}}$  is the z-transformed food application level calculated separately for the high and low food experiments.

CHAPTER 4  
ENERGY STORAGE STRATEGIES EXPLAIN DEVELOPING DISEASE VECTOR'S  
RESPONSE TO RISING TEMPERATURE

**4.1 Introduction**

Higher temperature increases the development rates of mosquitoes by speeding up biochemical reactions. Nonetheless, heightened reaction rates require more energy, and surprisingly little is known about how mosquitoes, and ectotherms in general, compensate energetically in order to mature faster in warmer conditions (Lafferty, 2009). It has been suggested that since organisms must allocate finite resources towards competing biological needs, temperature may affect the fitness tradeoffs between developing faster, meeting increased energy demand and maximizing size (Kozłowski et al., 2004). Such tradeoffs provide an attractive basis for the appearance of the temperature-size rule (TSR), the observation of decreased ectotherm size at higher temperatures. Indeed, despite being recognized as one of the most consistent rules in biology the TSR has eluded general, taxonomically widespread explanations (Atkinson and Sibly, 1997; Angilleta and Dunham, 2003).

For holometabolic ectotherms such as mosquitoes, temperature may affect the optimization of resource allocation between energy and growth in immature stages. Mosquitoes will be favored by avoiding depletion of energy reserves, which are involved in determining the timing of pupation (Gilpin and McClelland, 1979; Chambers and Klowden, 1990; Telang et al., 2007). Larval energy reserves have also been associated with adult flight ability (Nayar and Van Handel, 1971) and egg production (Zhou et al., 2004). Therefore, if mosquitoes are compelled to devote more resources towards energy storage instead of structural growth in order to compensate for increased metabolic activity at higher temperatures, final size may be reduced, thereby explaining the TSR. However, a temperature-induced reduction in body size is also likely to have fitness costs as larger mosquitoes have increased fecundity (Steinwascher, 1982;

Nakasathit and Scott, 1998; Ponlawat and Harrington, 2007), bite more persistently (Nasci, 1991), and survive longer (Reiskind and Lounibos, 2009). Clearly the effects of temperature on the growth-energy storage tradeoff demand attention; however, this issue has been scarcely addressed in part because historically, life-history models and empirical studies have assumed that energy storage and weight gain are directly proportional to one another in a temperature independent manner (Bertalanffy, 1960; Strong and Daborn, 1980; Berrigan and Charnov, 1994; Perrin, 1995). However, stored energy is but only one component of total mass, and if temperature were to affect the tradeoff between growth and energy storage, then it would produce changes in the proportion of total mass dedicated to energy storage.

Such an energy-growth tradeoff may be particularly important for mosquitoes that thrive in food limited habitats. Under food limitation energy stores may take on the additional role of bolstering short-term starvation resistance (Gilpin and McClelland, 1979), whereas structural growth may increase energy expenditure (Bertalanffy, 1960) and therefore susceptibility to food limitation. This takes on particular relevance for *Aedes aegypti* (L.), the major global vector of dengue fever, chikungunya and urban yellow fever. The principal larval habitats of this mosquito, domestic urban containers, are notoriously resource poor (Subra and Mouchet, 1984; Arrivillaga and Barrera, 2004; Strickman et al., 2003), and numerous field studies correlate the dynamics of adult production (Subra, 1983; Barrera et al., 2006b) and abundance (Southwood et al., 1972) with food limitation in the larval stage. Therefore, increased energy expenditure at higher temperatures may affect the ability of larval *A. aegypti* to obtain the resources necessary to mature in common urban habitats; this may add an additional pressure on developing larvae of this species to energetically compensate at higher temperature.

Here we model the potential tradeoffs between metabolism, energy storage and growth in *A. aegypti* larvae. In order to determine how rising temperature impacts these tradeoffs, we parameterized the model at 2°C intervals between 20 and 30°C, using laboratory experiments on weight gain, maturation time and starvation resistance. We use the model to determine the most likely mechanisms of resource allocation that give rise to the TSR in *A. aegypti* and their potential impact on the tradeoffs between size, starvation resistance and development rate as temperature increases. Using maximum likelihood model fits we make predictions of the impacts of rising temperature on energy reserves through the course of development and the rate of *A. aegypti* production in habitats with variable resource conditions.

## **4.2 Methods and Results**

### **4.2.1 Laboratory Experiments on Effects of Temperature on Development**

#### **4.2.1.1 Experimental conditions**

Temperature impacts on development were investigated by measuring development rate, growth and starvation resistance in larval *Aedes aegypti* (F3) from Barranquilla, Colombia (altitude 5 m, latitude 10 °N, mean monthly temperatures 26-29°C), using 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). In order to simulate natural conditions, the food used was a standardized household detritus collected in the vicinity of 30 water storage vessels in a dengue endemic city in Colombia. 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 standard laboratory foods used for mosquito rearing (Rueda et al, 1990).

In all feeding regimes, 100 mg of sediment was pre-incubated for 24 h in 0.9 L of water (in 1L feeding cups) in order to allow more time for micro-organism development as the sediment had been freeze dried. Ten newly hatched first instar larvae (L1) were added to each feeding cup, and another 100 mg of food was added after 48 h of feeding was completed. These feeding conditions had relatively small water volume in comparison to typical *A. aegypti* field habitats (Padmanabha et al, 2010) and very high food to larval ratio (albeit of an unknown food quality) in comparison to experimental studies of resource competition (Gilpin and McClelland, 1979; Jirakanjanakit et al, 2007); these conditions were chosen specifically in order to minimize resource competition and the time spent searching for food.

In order to measure the minimum feeding time required to commit to pupation (Experiment A) and starvation resistance (Experiment D) each larvae in four replicate vessels (n = 40) was placed in a 5 mL filled cup with distilled water and monitored daily until death or pupation. At each temperature this was 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. As a supplement to Experiment A, feeding was continued in four replicate feeding vessels at each temperature until the day in which 50% of the larvae had pupated, which we define as the median age of pupation ( $A_{pup}$ ). All observations on pupation and mortality were made on a daily basis, and no larval mortality in the feeding cups was observed.

In Experiment B we used the same feeding and temperature conditions to measure the dry weight trajectory at 28°C in larvae sacrificed daily after feeding from 0 to 4 days and incubation for 24 h in a drying oven. 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. After four days feeding dry weights were measured in individual larvae/pupae in three replicate cups (n=30). This latter procedure was carried out at all six temperatures in order to determine  $W_{L4}$ , the weight of late L4 or early pupae (Experiment C). Since  $A_{pup}$  was four days at 28°C the same 30 larvae were used in experiments B and C for this temperature. No larval mortality was observed in either of these experiments. Experiments B and C were conducted after Experiments A and D had terminated.

#### **4.2.1.2 Temperature effects on minimum feeding time required for commitment to pupation and median time to pupation (Experiment A)**

Feeding time required to pupate after transfer to distilled water decreased monotonically with increased temperature (Figure 4-1). All larvae that did not pupate incurred starvation mortality. Over 60% of larvae committed to pupation after 3 days feeding at 30°C, whereas at 20°C larvae needed to feed for 7 days in order to reach this pupation success (Figure 4-1). For larvae that committed to pupation, mean time (days  $\pm$ SD) to pupation after transfer to distilled water was for 30°C: 1.25 ( $\pm$  0.44), 28°C: 1.5 ( $\pm$  0.66), 26°C: 1.8 ( $\pm$  0.70), 24°C: 2.6 ( $\pm$  1.4), 22°C: 2.9 ( $\pm$  1.1) and 20°C: 5.4 ( $\pm$  2.4). These data indicate that transfer to distilled water stimulated larvae to initiate the physiological process of pupation, which proceeded faster with increasing temperature. At 24 and 20°C we observed outliers that initiated pupation after 5 days in starvation, but subsequently died before emerging as adults.

At 20, 22, 24, 26, 28 and 30°C our  $A_{pup}$  measurement was 9, 8, 7, 6, 5 and 5 days, respectively. The respective cumulative pupation percent at  $A_{pup}$  for each temperature was 30°C: 92.5%, 28°C: 60%, 26°C: 100%, 24°C: 82.5%, 22°C: 50%, 20°C: 52.5%. In these cups we did not record pupation percentage on a daily basis, only on the day in which we counted at least 20 pupae among the four feeding cups used to determine  $A_{pup}$ , at which point Experiment A was

discontinued. Other sources of uncertainty in our  $A_{pup}$  estimate include the course 24h time scale of observation, unknown sex ratios (time to pupation in 50% of the population may overestimate and underestimate pupation rate in males and females, respectively) and the fact that pupation percent was not recorded separately for each feeding cup. Nonetheless our  $A_{pup}$  estimate was similar to Rueda et al (1990), who recorded median time to pupation as 4.9 and 9.3 days at 30 and 20°C, respectively. This similarity is noteworthy, given important differences in the protocol of this study, including the use a laboratory colony of *A. aegypti*, 4-6 h interval between observations, a high protein homogeneous food source and a fixed 1:1 sex ratio.

#### **4.2.1.3 Dry weight trajectory at 28°C (Experiment B)**

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 between 3 and 4 days feeding (Figure 4-2). Larval instars after each feeding day were the following at 28°C: one day: L2, two day: L3, three and four days: L4. Over 75% of total weight was gained in the second half of development. This corresponds roughly to the finer scale observations of Telang et al (2007) in which 80% of total larval growth occurred in the L4 stage. The little growth from L1-L3 stages indicates that a sigmoidal function is a more accurate description of the data than a less than linear, exponential function.

#### **4.2.2.4 Temperature effects on $W_{L4}$ (Experiment C)**

At 20, 22, 24, 26, 28 and 30°C,  $W_{L4}$  was measured at 8, 7, 6, 5, 4 and 4 days, respectively. At each temperature this was a day prior to  $A_{pup}$ . We chose this measurement time, rather than weight at pupation for a number of reasons including: (1) pupation was not defined in our model fitting procedure (see below), (2) the large time interval (~24 h) between laboratory observations in comparison to the model time step (6 h) and (3) our model was not sex or stage specific. For simplicity in model fitting, these factors favored weight measurement in larvae at a

fixed age after the inflexion point in the tail end of the weight trajectory (Figure 4-2), rather than at a particular developmental stage. At 20 and 22°C  $W_{L4}$  was measured 2-days longer than the minimum feeding time required for at least 50% of larvae to commit to pupation, and one day longer at 24, 26 and 30°C (Figure 4-1). Given that only larvae in the middle of the L4 stage commit to pupation (Telang et al, 2007; Nishiura et al, 2007), we are confident that our  $W_{L4}$  estimation reflects the weight of late L4 that have surpassed the inflexion point in the sigmoidal weight gain trajectory (Figure 4-2) at these temperatures. Similarly Figure 4-2 indicates that weight increase after four days feeding at 28°C is likely to be small.

Mean  $W_{L4}$  decreased monotonically with increased temperature (Figure 4-3). This coincides with other studies that show a lower adult size/weight at higher rearing temperatures in *A. aegypti* (Rueda et al., 1990; Tun-Lin et al., 2000). From 20-28°C the effect of temperature, although monotonic with respect to the means, was small and of variable magnitude among 2°C intervals, with large overlaps in the 95% CIs among all treatments (Figure 4-3). At 30°C, however, there was a noticeable reduction in  $W_{L4}$ . As with  $A_{pup}$  these observations may be highly influenced by unknown differences in sex ratios among temperatures. We note that pupae were included in the average  $W_{L4}$  measurement in the following percentages of the total number of individuals (n=30) measured: 20°C: 3.3%, 22°C: 6.7%, 24°C: 47%, 26°C: 40%, 28°C: 3.3%, 30°C:10%. With the exception of 24 and 26°C, pupal weight was lower than the group means, suggesting that most of the pupae were males. However, differences in the proportion of pupae included in the  $W_{L4}$  measurement could be caused either by sex-ratios or slight non-linearities in the effects of temperature on developmental rate that may be accentuated due to our course, 24 h interval between observations. This is supported by the independent observation in Experiment

A of a high fraction that pupated at 24 (82.5%) and 26°C (100%) pupation at each respective  $A_{pup}$ .

#### 4.2.1.4 Temperature effects on starvation resistance through the course of development (Experiment D)

Starvation survival in the 40 larvae in each temperature-feeding treatment was characterized by obtaining maximum likelihood (ML) parameter estimates of a Weibull distribution of time to failure. The Weibull hazard rate is defined as:

$$h(t | k_{T,A}, \lambda_{T,A}) = (k_{T,A} / \lambda_{T,A})(t / \lambda_{T,A})^{k_{T,A}-1}, \quad (4-1)$$

where  $h(t | k_{T,A}, \lambda_{T,A})$  is the hazard of death of larvae in each time period (t) subsequent to transfer to distilled water, given the estimated parameters of the Weibull distribution ( $k_{T,A}$ ,  $\lambda_{T,A}$ ),  $k_{T,A}$  is the shape parameter and  $\lambda_{T,A}$  is the scale parameter, both of which were estimated via ML analysis of observed survival in the 40 larvae starved in each temperature (subscript T) and feeding time (subscript A) treatment. Under a constant shape parameter ( $k$ ), increases in the scale parameter ( $\lambda$ ) will push the distribution towards the right and flatten  $h(t)$ , whereas decreasing  $\lambda$  raises the distribution and pushes it to the left. Graphically,  $\lambda$  is found at the time with the maximum hazard of death and is therefore referred to as the characteristic life. By contrast, the shape parameter describes the trajectory of the hazard over time (decreasing to zero if  $<1$ , increasing to infinity if  $>1$ ), which in these experiments is expected to increase as starved larvae have increasingly fewer energy reserves to maintain basal metabolism ( $k > 1$ ). The maximum feeding day from each temperature whose survival data was employed for model fitting had at least 10 larvae that experienced starvation mortality without pupating. This was 3, 4, 4, 4, 5 and 6 for 30, 28, 26, 24, 22 and 20°C respectively.

Since the accumulation of sufficient energy reserves determines both pupation and starvation resistance (Gilpin and McClelland, 1979; Telang et al., 2007), we assumed that larvae that pupated in distilled water had higher energy reserves upon removal of food than those that did not pupate. Therefore ML estimates of the Weibull survival (scale parameter) assumed that all larvae that pupated after being placed in distilled water are right censored at the starvation time of the longest surviving larva that did not pupate. This assumption overlooks other factors, such as overall weight or genetic factors that may also contribute to starvation resistance. By contrast, interval censoring would have assumed that larvae that pupated had the same hazard as those that did not, which is clearly contrary to the evidence linking energy reserves and pupation. Alternatively, mean starvation resistance estimates exclude pupae, which are more likely to be males, and would have considerably reduced sample size in many cases. Although we know of no studies that examine sexual dimorphism in starvation resistance in mosquito larvae, because males accumulate lipids more efficiently and weigh less than females (Timmermann and Briegal, 1999), they may have heightened starvation resistance. Thus, while none of these alternatives are ideal we considered that treating pupae as right censored was the best representation of starvation resistance in the original 40 larvae cohort.

In unfed larvae, starvation resistance, as measured by the Weibull scale parameter ( $\lambda$ ), decreased as temperature rose above 22°C; however, it dropped off steeply at 20°C, which may approach the cold tolerance of the tropical, sea level *A. aegypti* strain used (Figure 4-4). Figure 4-4 also depicts changes in relative starvation resistance of unfed vis-à-vis 1-day fed larvae across temperatures. At 20 and 22°C starvation resistance was similar or slightly higher in unfed as compared to 1-day fed larvae. At 24 and 26°C, survival was lower after one day of feeding

with no overlap in the 95% CIs of  $\lambda$  at 24°C and only a slight overlap at 26°C (Figure 4-4). At 28 and particularly 30°C, however, survival was clearly higher in the 1-day fed group (Figure 4-4).

In general, the data support the logical assumption of an increased risk of mortality as the starvation period increases and larval energy reserves are depleted, with  $k > 2$  for virtually all temperature-feeding treatments. The exception to this pattern occurred in 1-day fed larvae at 20 and 22°C, which had a bimodal hazard, with high numbers of larvae dying both at the beginning and at the end of the starvation period. This generated the large confidence intervals for the scale parameter in these treatments (Figures 4-4 and 4-5) and is also reflected in the shape parameter estimate in 1-day fed larvae at 20°C (1.3, 95% CI: 1.0, 1.6) and 22°C (0.78, 95% CI: 0.61, 0.98), much lower in comparison to all other feeding and temperature regimes. For example, the shape parameter (95% CI) for starvation resistance in 1-day fed larvae in the other temperatures was 3.1 (2.5, 3.9) at 24°C, 3.3 (2.6, 4.1) at 26°C, 5.5 (4.2, 7.0) at 28°C and 6.1 (4.9, 7.7) at 30°C.

A rise in starvation resistance was observed over the course of development, consistent among temperature treatments (Figure 4-5). In 1-day fed larvae starvation resistance was approximately constant across temperatures. In the 2-day fed group  $\lambda$  was 7.6 (6.9-8.4) at 24°C and 8.5 (7.9-9.1) at 26°C, in comparison to 10.1 (9.5-10.7) at 22°C, 9.6 (9.1-10.1) at 28°C and 11.0 (10.1-12.0) at 30°C (Figure 4-5). In the 3 and 4-day fed groups, starvation resistance was clearly higher in the three warmest temperatures (Figure 4-5). It should be noted that right censoring for pupae occurred in the Weibull survival estimate (Figure 4-5) on the final feeding day at each temperature except for 24°C. At this temperature no pupae were observed in the group starved after 4-days feeding, but in the 5-day fed group 38 of 40 larvae (95%) pupated after transfer to distilled water (Figure 4-1).

#### 4.2.2 Model of Growth and Energy Storage in *Aedes aegypti* (L.)

We developed a model of *A. aegypti* larval development that simulated growth and energy storage under our experimental conditions in order to achieve following objectives: 1) use the model to explore potential tradeoffs between development rate, size, starvation resistance and energy reserves; 2) use the experimental data to determine the temperature-dependence of model parameters and the sensitivity of the model fit to parameter variation; 3) use the fitted model to predict temperature impacts on energy reserves and pupation rate under a continuum of food scenarios.

Through a set of discrete time equations, the model tracks larval weight and energy storage until pupation in individual larvae. Food assimilated into biomass may be partitioned into structural growth, stored energy or basal metabolic needs (Kozłowski et al., 2004). Metabolic costs, which depend on weight, are paid first (Kozłowski et al., 2004), and thus excess resources are allocated between growth and energy storage, both of which contribute to weight gain. As evidenced in prior studies, energy stores determine both starvation survival and the timing of pupation (Wigglesworth, 1942, Gilpin and McClelland, 1969; Telang et al, 2007). Based on the hypothesized tradeoff between energy storage and size, we also model the rate of food assimilation as a function of energy stores. Therefore a change in energetic requirements and/or food assimilation efficiency may affect growth rate directly or indirectly by modifying how larvae optimize the allocation of resources between structural growth and energy storage. We estimated and constrained parameters for the model using the results of Experiments A-C, simulated growth and starvation in individual larvae, and then assessed the fit of predicted starvation resistance to temperature and age specific data from Experiment D. Given larval development times on the order of 5-9 days (Rueda et al, 1990) we considered 6 h as a biologically reasonable time step for tracking *A. aegypti* growth and energy reserves (Gilpin and

McClelland, 1979). Simulated growth and survival were summed over 24 h intervals in order to compare model outputs with daily experimental observations.

#### 4.2.2.1 Nutrient assimilation into biomass

For simplicity, larvae do not incur metabolic costs for searching or handling food, corresponding roughly to our experimental conditions (see above). In mosquitoes and *A. aegypti* in particular, our own data (Figure 4-2) and numerous other studies indicate a sigmoidal growth trajectory, with a burst in growth at approximately the molt to fourth instar (Dye, 1984; Nishiura et al., 2007; Telang et al., 2007). Equation 4-2 describes the rate of nutrient assimilation when nutrients are not limiting. In accordance with the sigmoidal weight trajectory, nutrients assimilated [hereby referred to as food assimilated] ( $F_{A,i,t}$ ) for each larva  $i$  of feeding age  $A$  per unit time are assumed to take on a Gaussian function over the course of development when larvae are assimilating food at their physiological capacity.

$$F_{A,i,t} = gW_{i,t}e^{-[(E_t - E_{th})^2 / 2\sigma^2]} \quad (4-2)$$

Under unlimited food conditions, such as those assumed in our feeding experiments used to parameterize the model, the food available to larvae is greater than the right side of Equation 4-2. In later simulations we use the model to predict the impacts of temperature in food limiting conditions, in which food assimilated may be less than this quantity.

The proportion of total body weight ( $W_{i,t}$ ) that can be assimilated into biomass when food assimilation is at a maximum is termed maximum growth efficiency ( $g$ ). The key assumption that links growth and energy stores over the course of development is that the location of the “hump” in assimilation rate is triggered by a threshold level of energy stores ( $E_{th}$ ). Therefore increasing energy stores before reaching  $E_{th}$  will tend to *increase* food assimilated, whereas

energy storage after  $E_{th}$  (during the latter part of the L4 stage) will *decrease* food assimilated. This assumption is supported by Timmermann and Briegel (1999), whose experimental work showed decreasing lipid assimilation rate in the last instar of *A. aegypti*, which according to Equation 4-2, would increase growth in the fourth instar as  $E_{i,t}$  would remain in the vicinity of  $E_{th}$ . Moreover, Telang et al (2007) showed that larval weight upon molting to the last instar is approximately 20% of the final weight attained under optimal feeding conditions; this suggests that a sigmoidal function is a better description of weight trajectory than an exponential or linear to plateau-type shape. The spread parameter ( $\sigma$ ) determines the trajectory of increase and decrease of the food assimilation rate as  $E_{i,t}$  approaches and surpasses  $E_{th}$ .

*Weight* Equation 4-3 describes weight ( $\mu\text{g}$ ) dynamics of food assimilated into body mass and the loss due to consumption of energy reserves in order to meet basal metabolic needs.

$$W_{i,t+1} = W_t + F_{A,i,t} - c_m (W_{i,t})^m \quad (4-3)$$

The parameter  $c_m$  is the coefficient of metabolic expenditure as a proportion of larval weight ( $W_{i,t}$ ) scaled by  $m$ , the exponent describing weight dependence of metabolism or metabolic allometry (Bokma, 2004). These parameters are roughly analogous to those describing catabolism in the original Bertalanffy (1960) model. However, as customary in models optimal resource allocation (Kozłowski et al, 2004), weight is lost only under conditions when food assimilated does not meet the energetic demands of basal metabolic functions (Eq. 4-5).

#### 4.2.2.2 Stored energy dynamics

Allocation of resources towards stored energy, represented in units of mass ( $\mu\text{g}$ ), is described in Equation 4-4 when food assimilated is greater than the minimum metabolic requirement. Equation 4-5 describes the expenditure of stored energy when food assimilated

does not cover metabolic requirements. Larval death from starvation occurs in the time interval immediately after energy reserves are depleted to zero.

$$E_{i,t+1} = E_t + c_s [F_{A,i,t} - c_m (W_{i,t})^m]^s \text{ if } (F_{A,i,t} > c_m (W_{i,t})^m) . \quad (4-4)$$

$$E_{i,t+1} = E_t + F_{A,i,t} - c_m (W_{i,t})^m \text{ if } (F_{A,i,t} < c_m (W_{i,t})^m) . \quad (4-5)$$

The efficiency of energy storage ( $c_s$ ) represents the proportion, scaled by the exponent  $s$ , of food assimilated above the minimum metabolic requirements that a larva allocates towards energy storage. Although not formally tracked in the model, this formulation assumes that all food assimilated that is not allocated towards energy stores is devoted to structural growth, such that total weight ( $W_{i,t}$ ) is the sum of energy reserves ( $E_{i,t}$ ) and structural mass ( $S_{i,t}$ ):

$$W_{i,t} = E_{i,t} + S_{i,t} . \quad (4-6)$$

We model energy storage as a stochastic process with independent trials for each 0.1  $\mu\text{g}$  of food assimilated. In mosquitoes energy reserves may take the form of carbohydrates, lipids or amino acids, each of which may vary with different food sources (Wigglesworth, 1942; Nayar and VanHandel, 1971; Timmermann and Briegel, 1999). For simplicity, given the heterogeneous, field collected detritus used in the experiments, we assume that each particle of food assimilated has the same probability of being converted into energy reserves. The probability that each piece of assimilated food (Eq. 4-2) is allocated towards energy stores in (Eq. 4-4) is therefore assumed to take on a binomial distribution with a probability  $p$  equal to the coefficient of energy storage ( $c_s$ ) and a number of trials  $n$  equal to the scaled amount of food assimilated above maintenance costs  $(F_{A,i,t} - c_m W^m)^s$ , within every 6 h interval. Given our assumption that all food assimilated has the same potential to be converted into energy reserves,

the value of the parameter  $c_s$  represents the optimal proportion of excess food assimilated at a particular weight that a larva allocates towards energy storage instead of structural growth.

#### **4.2.2.3 Pupation**

Pupation is modeled in latter simulations in which we use the model to predict development rate under an array of feeding conditions. Stored energy is assumed to trigger pupation (Gilpin and McClelland, 1979; Klowden and Chambers, 1990; Timmermann and Briegel, 1999; Nishiura et al., 2007; Telang et al., 2007), such that larvae pupate when stored energy reaches  $E_{pup}$ . Unlike other studies of *A. aegypti* (Chambers and Klowden, 1990) we do not assume a threshold, or critical weight required for pupation, as this is unlikely to be directly involved in the physiological mechanism of pupation and may also vary with food availability (Gilpin and McClelland, 1979; Nishiura et al, 2007). Although critical weight has been shown to increase with lower temperature (Chambers and Klowden, 1990) the relationship between  $E_{pup}$  and temperature has not been studied to our knowledge. Therefore, we did not make any temperature assumptions with regards to  $E_{pup}$ , *per se*, although, based on the data on commitment to pupation (Experiment A, Figure 4-1), we did assume that the rate of accumulation of energy reserves increased with temperature (see model fitting section below). An additional criterion for pupation is a temperature-dependent minimum development age  $A_{pup}$  (Experiment A). Larvae that reach  $E_{pup}$  will only pupate if they have also completed  $A_{pup}$ , whereas larvae that reach  $E_{pup}$  after  $A_{pup}$  pupate at the beginning of the next time interval.

#### **4.2.3 Parameter Estimation and Model Fitting Using Experimental Data**

##### **4.2.3.1 Summary of model fitting strategy and assumptions of temperature effects on parameters**

The model fitting process consisted of specifying and constraining parameters in order to match Experiments A, B, C and starvation resistance of unfed larvae, and then using ML analysis to

assess the fit of the model to Experiment D, separately for each temperature. Therefore, model fits to Experiment D are all predicated on the observations that: (1) rising temperature induces commitment to pupation with a shorter feeding time (Experiment A, Figure 4-1), (2) at 28°C, larval weight gain in optimal feeding conditions follows a sigmoidal trajectory (Experiment B, Figure 4-2), (3) the TSR applies in *A. aegypti* with a monotonically decreasing  $W_{L4}$  as temperature rises from 20-30°C (Experiment C, Figure 4-3) and (4) starvation resistance of unfed larvae decreases as temperature rises from 22 to 30°C (Figure 4-4). Because pupation requires the accumulation of sufficient energy reserves and starvation mortality occurs when energy reserves are depleted, the model also assumes, based on observations (1) and (4) above, that energy reserves rise faster in feeding larvae and are spent faster in starved larvae as temperature rises. Accordingly our parameter estimates, sensitivity analyses and model predictions suggest how the observed temperature effects on *A. aegypti* development are brought about and the implications for temperature effects across a spectrum of resource conditions. Implicitly, we assume that neither age nor food limitation affects growth and metabolic parameters.

Mathematical explanations of the TSR suggest that temperature may affect the coefficients and/or exponents of catabolism and anabolism, which are usually modeled as the processes that define the rate of growth (Bertalanffy, 1960; Berrigan and Charnov, 1994; Perrin, 1995; Angilleta and Dunham, 2003; Angilleta et al., 2004; Kozłowski et al., 2004). Similarly we also assume that temperature may affect the coefficients ( $c_m$  and  $c_s$ ) and/or exponents ( $m$  and  $s$ ) of catabolic and anabolic activity, with the key difference that in our model these processes determine the dynamics of stored energy, which only partially contributes to total mass and also affects food assimilation rate. We also assume that temperature may affect maximum food assimilation efficiency ( $g$ ), an assumption well supported in ectotherms (Angilleta and Dunham,

2003) and in *A. aegypti* in particular (Rashed and Mulla, 1989). In our model  $g$  determines the peak of the Gaussian food assimilation rate whereas the other two parameters involved in the growth trajectory,  $E_{th}$  and  $\sigma$ , determine how the feeding rate varies with energy reserves. For simplicity in the absence of evidence to the contrary, we assume that both  $E_{th}$  and  $\sigma$  are temperature independent. These assumptions regarding the temperature dependence of model parameters suppose that temperature may directly affect weight gain by affecting the overall efficiency of food assimilation or indirectly by affecting the rate of consumption or build-up of energy reserves; however, the assumed temperature independence of  $E_{th}$  and  $\sigma$  means that temperature will not affect how food assimilation responds to a particular level of stored energy. We divide the parameters (Table 4-1) into four categories: initial conditions ( $W_o, E_o$ ), assumed temperature independent parameters ( $\sigma, E_{th}$ ), temperature dependent parameters specified to match experimental data ( $c_m, s$ ), and temperature dependent parameters evaluated through ML model fits to observed starvation survival and 28°C weight trajectory after successive feeding days ( $g, m, c_s$ ). Table 1 summarizes the method of estimation of each parameter.

#### **4.2.3.2 Initial conditions ( $W_o, E_o$ )**

Initial weight ( $W_o$ ) was assumed to be the same across larvae and set to the mean of the group means measured in newly hatched larvae (unfed larvae in Experiment C). Since no information was available on the energy stores present in newly hatched mosquito larvae ( $E_o$ ), we arbitrarily assigned an average  $E_o$  equal to 40% of  $W_o$ .

Genetic variability and variation in egg resources are two potential sources in which  $W_o$  or  $E_o$  may vary among larvae. However since weight in newly hatched larvae was not individually measured (due to the limits of our weighing device), the only information we had that would allow us to infer variation in initial conditions was in the starvation survival of newly hatched larvae. Because starvation resistance involves depletion of energy reserves, we

incorporated this variation into the assignation of initial energy reserves for each larva ( $E_o$ ). Using Equation 4-5, we chose  $c_m$  so that stored energy of a starved, newly hatched L1 would reach zero at a time corresponding to observed mean starvation resistance at each temperature. Weight dependence of metabolism, represented in the exponent  $m$ , was set at 2/3 the value assumed in prior *A. aegypti* models (Gilpin and McClelland, 1979). [However, this assumption was later relaxed when the model fit was compared to experimental data at all six temperatures (see below).] Using  $\lambda_{T,A}$  and  $k_{T,A}$ , estimated based on starvation of unfed larvae in Experiment D (Figure 4-4, Table 4-1), we generated a distribution of starvation survival time upon hatching among 100 larvae in each of the six temperatures. Having assumed  $m$  and fit  $c_m$  this enabled us to use Equation 4-4 to iteratively generate an  $E_o$  value for each simulated larva. Histograms of the 600  $E_o$  fits were then employed to construct a temperature-independent distribution of initial energy reserves. From this distribution we randomly assigned  $E_o$  to each larva in all subsequent simulations.

#### **4.2.3.3 Temperature dependent parameters chosen to match data ( $c_m$ , $s$ )**

As described above, the coefficient of metabolism ( $c_m$ ) was chosen using Equation 4-4 to match *mean* starvation survival of the 40 newly hatched larvae observed at each temperature (unfed larvae in experiment D), for a given value of  $m$ . This approach, instead of choosing  $c_m$  for each individual larva, ensured that  $c_m$  and  $E_o$  were not coupled as an artifact of the fitting process. Figure 4-6 gives the joint  $c_m$  versus  $m$  parameter space that reproduced starvation resistance of newly hatched larvae at each temperature. Starvation resistance of newly hatched larvae is much more sensitive to the coefficient than the exponent of metabolism, such that at the most common ranges of the exponent  $m$  evoked by ecologists (0.5-1) (Bokma, 2004; Irlich et al., 2009)  $c_m$  must take on a narrow range between 0.02 and 0.08 at all temperatures. The

interdependence of  $c_m$  and  $m$ , given by the slope of the curves in Figure 4-6, roughly decreases with lower temperature and higher values of  $m$ .

For a given  $m$  value, the relative value of  $c_m$  among temperatures corresponds inversely to the relative starvation survival of unfed larvae among temperatures (Figure 4-4); that is, the fit value of  $c_m$  increases as temperature rises from 22-30°C (Figure 4-6). At 20°C  $c_m$  lies between the 26 and 28°C estimates (Figure 4-6). Our approach to fitting  $c_m$  was based on the assumption that the differential survival of unfed larvae among temperatures reflected differences in the energetic costs of metabolic maintenance. However, increased metabolic requirements is an unlikely cause of higher mortality of unfed larvae at 20°C as compared to 22-26°C (Figure 4-4). Genetic factors related to the cold tolerance of the *A. aegypti* strain used are a more likely cause, but not accounted for in the model.

We randomly assigned a value for  $W_{L4}$  to each simulated larva, based on histograms generated from the 30 larvae weighed in each temperature (Experiment C). The simulation chose a value of  $s$  for each larva so that it would comply with its assigned  $W_{L4}$ . This exponent determines whether energy allocation levels off ( $s < 1$ ), is proportional to ( $s = 1$ ), or increases ( $s > 1$ ) with more food assimilated in the latter portion of the L4 stage; we considered it biologically unreasonable for energy storage to decrease with increased weight, and thus model fitting was constrained to non-negative  $s$  values.

#### **4.2.3.4 Temperature independent parameters specified ( $E_{th}, \sigma$ )**

Because we were unable to find studies that describe feeding efficiency or the trajectory of energy reserves in early larval instars, we arbitrarily specified  $E_{th}$  as approximately ten times  $E_0$ , or 15  $\mu\text{g}$ . We subsequently assumed a  $\sigma$  value that produced a mean larval weight trajectory that was roughly consistent (visually) with observed group mean weight of larvae fed for 1, 2 and 3 days at 28°C (Experiment C). For this analysis the metabolism exponent ( $m$ ) was varied

from 0.5 to 0.75 (Bokma, 2004), as opposed to assuming the 0.66 value that was used to estimate variation in  $E_o$ . We made this modification since weight trajectory is temperature dependent, and a number of studies have suggested temperature dependence in the catabolism exponent (Angilleta and Dunham, 2003; Kozłowski et al, 2004). Growth efficiency ( $g$ ) was varied from 0.3 to 0.8 and the energy storage coefficient ( $c_s$ ) from 0.1 to 0.4; the metabolism coefficient  $c_m$  and energy storage exponent ( $s$ ) were chosen to match mean starvation survival of unfed larvae and  $W_{L4}$  at 28°C, respectively, given the value of  $m$ ,  $g$  and  $c_s$  in each trial. This analysis produced similar weight trajectory to observed data when the  $\sigma$  value was in approximately the 17-28  $\mu\text{g}$  range. This large “spread” ( $\sigma$ ) in the Gaussian curve relative to the “mean” ( $E_{th}$ ) indicates a gradually increasing and decreasing food assimilation rate as energy reserves approach and surpass  $E_{th}$ . By contrast small values of  $\sigma$  would have produced a low food assimilation rate at the beginning of development and a steep rise and drop in the vicinity of  $E_{th}$ . Using this information we assumed  $\sigma = 23$  and  $E_{th} = 15 \mu\text{g}$  for all temperatures throughout the rest of the process of model fitting and generation of predictions.

#### **4.2.3.5 Estimation of temperature-dependent parameters ( $m$ , $c_s$ , $g$ ) through ML comparison of model to experimental data**

We obtained ML estimates for the metabolic exponent ( $m$ ), maximum growth efficiency ( $g$ ) and the coefficient of energy storage ( $c_s$ ) by comparing starvation resistance in larvae fed successively for one to six days (Experiment D) to that of larvae simulated using Equations 4-2 to 4-5. At 28°C this comparison was constrained to parameter spaces that also maximized the likelihood of the observed weight trajectory (Experiment B). At all other temperatures the parameter space was constrained to ensure a faster rate of increase in energy reserves with increasing temperature, consistent with our data on commitment to pupation (Experiment A).

In order to fit the model to experimental data on starvation resistance at each feeding age we used the following temperature-specific function:

$$LL_{H,T} = \sum_{F=1}^{A_{max}} \sum_{i=1}^n \ln pr(t = t_i | \lambda_{T,A}, k_{T,A}), \quad (4-7)$$

where  $LL_{H,T}$  is the log-likelihood that death hazard of simulated larvae follows the Weibull probability density function estimated from observed data at temperature  $T$ ,  $t_i$  is time to death from starvation of each simulated larva, assumed to occur at the beginning of the time interval after energy reserves fall to zero,  $n$  is the number of larvae in each temperature-feeding age,  $F$  is feeding age – the days reared from hatching in feeding cups prior to starvation and  $A_{max}$  is the maximum feeding age that was starved at each temperature. This formulation summed the Weibull probabilities of the predicted time of death of each simulated larva, based on the observed  $\lambda_{T,A}$  and  $k_{T,A}$ . ML fits maximized the mean  $LL_{H,T}$  among 100 simulations ( $n = 40$  larvae in each) for each parameter combination.

In order to ensure that the model fits complied with the observed weight trajectory, ML estimates of  $g$ ,  $m$  and  $c_s$  at 28°C involved maximizing the sum  $LL_{H,28} + LL_{W,28}$ , where the latter term refers to the likelihood that simulated larvae comply with the observed weight trajectory from 1 to 3 days feeding (weight of the 4-day fed group was used to fit  $s$ ) (Experiment B). Since weight was measured at the group level for these treatments, we determined the likelihood that the observed mean and variance in weight *among* groups described the distribution of group weights in a pool of simulated larvae that were randomly resampled and placed into groups of the same size used for weighing. This is summarized in the following log-likelihood function:

$$LL_{W,28^\circ} = \sum_{d=1}^3 \sum_{i=1}^{40} \ln pr(x = \hat{x}_i | \mu_{g,d}, \sigma_{g,d}^2), \quad (4-8)$$

where  $\hat{x}_i$  is the total weight of group  $i$ , consisting of  $n$  resampled larvae ( $n=5$  for 1-day fed, 4 for 2-day fed and 2 for 3-day fed larvae),  $\mu_{g,d}$  is the observed mean weight among groups of larvae after  $d$  feeding-days and  $\sigma^2_{g,d}$  is the observed variance.

At all other temperatures (20, 22, 24, 26 and 30°C) the estimation of  $g$ ,  $m$  and  $c_s$  (through maximization of  $LL_{H,T}$ ) was constrained to ensure compliance with commitment to pupation (Experiment A). This was achieved by first fitting the model at 28°C and then assuming that that the minimum percentile that pupated at a higher temperature had on average (among 100 simulations) more energy reserves than the *maximum* percentile that did *not* pupate at a lower temperature (Figure 4-1). Energy storage constraints for 30, 26 and 24°C were defined based on the ML 28°C scenario, at which parameters were independently estimated based on both weight trajectory and starvation survival. For example, in Experiment A among larvae starved after four days feeding, 92.5% pupated at 30°C, 72.5 % pupated at 28°C, 50% pupated at 26°C and none pupated at 24°C (Figure 4-1). Therefore, at 26°C the joint  $g$ ,  $m$  and  $c_s$  parameter space was constrained such that the average value of the 50<sup>th</sup> percentile in energy reserves after four days feeding was lower than the average 22.5<sup>th</sup> percentile ML value at 28°C; at 24°C we considered only the parameter space in which the average value of the highest energy reserves after four days feeding was lower than the 22.5<sup>th</sup> percentile value at 28°C, and at 30°C the 7.5<sup>th</sup> energy percentile was constrained to be higher than the 22.5<sup>th</sup> percentile value at 28°C. Because of the large gap between time to commitment to pupation between high and low temperatures, constraints at 22°C were defined based on ML 24°C estimates and 20°C constraints were based on ML 22°C estimates. Pupation was not allowed in the model fits to Experiment D, as  $E_{pup}$  was fit *a posteriori* based on the predicted energy trajectories (see model prediction section).

At all temperatures we conducted first a likelihood analysis of the full range of the joint  $m$ ,  $c_s$  and  $g$  space, varying each at 0.05 intervals between 0.05 and 1 (i.e. 8000 parameter combinations). A narrow range, joint ML parameter space was defined by taking the two values for each parameter that yielded the highest overall likelihood among the 8000 combinations, and adding 0.05 to the higher value and subtracting 0.05 from the lower value. This yielded a joint parameter space of 0.15 for all three parameters at all temperatures, except for the metabolism exponent ( $m$ ) at 20°C and growth efficiency ( $g$ ) at 24°C, both of which had an ML narrow range space of 0.2 (Table 4-2). The definition of this narrow range was not constrained by the assumption of energy reserves with respect to Experiment A, as described above.

We subsequently carried out a likelihood analysis over the narrow range ML parameter space (Table 4-2), varying each of the three parameters at 0.01 intervals (i.e. 4096 parameter combinations at 22, 26-30°C and 5376 combinations at 20 and 24°C). This was repeated 100 times for each temperature, constraining the analysis only to ML parameter combinations that were compliant with the energy reserves assumption regarding pupation in Experiment A. The estimates of  $g$ ,  $m$  and  $c_s$  used to generate model predictions was considered the mean of the 100 ML values generated for this narrow range analysis and 95% CIs were set at the 2.5 and 97.5 percentiles (Figure 4-7A). The metabolic coefficient  $c_m$  was assumed constant among all simulated larvae and was fit at each temperature to reproduce observed mean starvation resistance in unfed larvae, given the ML estimate of  $m$ . The energy storage exponent  $s$  was fit to match the randomly assigned  $W_{L4}$  of each simulated larva, given the ML estimates of  $g$ ,  $m$  and  $c_s$  and the fit of  $c_m$ . Mean and SD of the fit value of  $s$  among individual larvae are reported in Figure 4-7B. The values of  $E_o$ ,  $\sigma$  and  $E_{th}$  were assumed as described above (Table 4-1).

Log-likelihood estimates ( $LL_{H,T} + LL_{W,28}$  for 28°C and  $LL_{H,T}$  for all other temperatures) were the sum of 40 larvae fed until  $A_{max}$  at each temperature, averaged over 100 simulations for each parameter combinations (Equations 4-7 and 4-8). Averaged maximum likelihood estimates were the following: 20°C: -553.0 ( $A_{max} = 6$ ), 22°C: -472.9 ( $A_{max} = 5$ ), 24°C: -332.0 ( $A_{max} = 4$  days), 26°C: -329.5 ( $A_{max} = 4$ ), 28°C: -555.1 ( $A_{max} = 4$  plus 1-3 days feeding for weight) and 30°C: -219.7 ( $A_{max} = 3$ ). Likelihoods were increasingly negative in those temperatures with more data included (Bolker, 2008). For example  $LL_{H,28°C} + LL_{W,28°C}$  summed 280 larvae as compared to only 90 larvae for  $LL_{H,30°C}$ . Maximized log-likelihoods, standardized per larva, were as follows: 20°C: -2.30, 22°C: -2.36, 24°C: -2.07, 26°C: -2.05, 28°C: -1.98 and 30°C: -1.83. This indicates a slightly less precise model fit at 20 and 22°C.

ML estimates of growth efficiency ( $g$ ) demonstrate an approximately linear increasing relationship with temperature (Figure 4-7A). The metabolic coefficient ( $c_m$ ) decreases from 20 to 22°C (due to the low observed survival of newly hatched larvae at 20°C), and increases monotonically with temperature at 22-30°C, with an increasing slope as temperature rises (Figure 4-7A). Energy storage coefficient ( $c_s$ ) is roughly constant from 20 to 26°C, but shows a sharp increasing trend from 26-30°C (Figure 4-7A). The mean estimate of the energy storage exponent ( $s$ ) (fit to individual larvae) increases until 26°C and fall dramatically at 28°C, the temperature at which  $c_s$  increased (Figure 4-7B). Estimates of  $s$  are clearly higher at 24 and 26°C as compared to the other temperatures. From 22-30°C, the exponent of metabolism ( $m$ ) fluctuates from 0.5 to 0.58, at the lower end of the 0.5-0.75 range commonly cited by ecologists. At 20°C, however, the value of  $m$  is much lower and inconsistent with the literature (Bokma, 2004). This is an artifact of the joint dependence of  $m$  and  $c_m$  to starvation resistance of unfed larvae (Figure 4-6), which was unexpectedly low at 20°C (Figure 4-4), resulting in a large fit value for  $c_m$  in

comparison to 22°C (Figures 4-6 and 4-7A). Notably, across all temperatures  $m$  is significantly smaller than  $s$ , indicating that while energy storage varies close to linearly with increasing food assimilated, as overall weight increases, basal metabolic demands are proportionally lower (Eq. 4-4 and Eq. 4-5).

Figure 4-8 depicts the comparison of simulated and observed mean weight trajectory at 28°C and starvation resistance at all temperatures, using parameter values shown in Figure 4-7. In order to directly compare simulated and observed means in starvation treatments with pupation, simulated means in Figure 4-8 were calculated without the longest surviving larvae in each temperature-food category, corresponding to the number of observed larvae that pupated in starvation in Experiment A (i.e. percent of larvae truncated in each treatment corresponds to pupation percentage in Figure 4-1). Figure 4-8 shows that using ML parameter estimates, the model on the whole adequately reproduced weight trajectory at 28°C (Experiment B) and starvation resistance at each temperature (Experiment D), with simulated mean survivals located well within observed standard deviations and vice versa. Slight parameter changes (not shown) within the narrow ML ranges (Table 4-2) permitted a closer fit between observed and simulated means at all temperatures, but these shifts generated consistently lower likelihoods, attesting to the differences between mean survival and the Weibull hazard function. Deviations between simulated and observed data are particularly evident at 20°C. In concert with increased confidence intervals for estimates of both  $m$  and  $c_s$  (Figure 4-7), this further calls into question the biological validity at 20°C of our assumption equating starvation resistance of unfed larvae with basal metabolic parameters  $c_m$  and  $m$ .

## 4.2.4 Sensitivity Analyses

### 4.2.4.1 Effects of parameters on weight, starvation survival and energy reserves

In order to characterize tradeoffs between growth, starvation survival, the mass of energy reserves and the proportion of weight dedicated to energy reserves ( $E/W$ ) in feeding larvae, we determined the independent effect of each parameter on each of these four outcomes. This analysis focused on the behavior of the deterministic model in which stochastic energy storage was eliminated; thus,  $c_s$  represented the proportion of all food assimilated into biomass in each 6hr time step that went towards energy storage as opposed to structural growth (Equation 4-6). We also excluded individual variation in  $E_o$  and  $W_{LA}$ , simulating one larva representing mean conditions. All temperature dependent parameters were independently varied to  $\pm 100\%$  of their 28°C ML estimate in order to assess their effect on model behavior beyond the range of ML parameter estimates, and all model fitting constraints to ensure consistency with Experiments A-C were also removed. The values of  $E_o$ ,  $E_{th}$  and  $\sigma$  assumed in Table 4-1 were maintained. Since individual variation was removed in this analysis, we used as the baseline value of  $s$  the mean value given in Figure 4-7B.

Overall, maximum growth efficiency ( $g$ ) (green curve in Figures 4-9 to 4-12), had a strong positive effect on weight gain (Figure 4-9) but had little impact on starvation resistance (Figure 4-10). In contrast, the parameters describing basal metabolic activity ( $c_m$  and  $m$ , red shades) had a large negative impact on starvation resistance, with little impact on weight gain. Increases in energy storage parameters ( $c_s$  and  $s$ , blue shades) clearly cause a tradeoff between growth and starvation resistance, by favoring starvation resistance throughout development but inducing a sharp *decline* in size after 3 and 4 days feeding (Figure 4-9C and 4-9D, Figure 4-10). Interestingly, weight after 4 days feeding shows more sensitivity to energy storage parameters (negative relationship) than growth parameters (positive relationship) (Figure 4-9D). This

indicates that in the late fourth instar, the *indirect* effect on weight gain of increased energy storage on reducing food assimilated outweigh the direct effects of heightened growth efficiency (Equation 4-2). These results indicate that relative to the 28°C parameter estimates, reduced size at higher temperatures cannot be explained the effects of temperature on the metabolic parameters ( $c_m$  and  $m$ ) in *A. aegypti*, as postulated by Perrin et al (1994). Moreover, since higher temperature increases the maximum food assimilation efficiency ( $g$ ) (Figure 4-7A), reduced size at higher temperatures in the vicinity of ML parameter estimates can only be attained through increased allocation of resources towards energy stores. At lower temperatures reduced energy storage parameters will increase food assimilation in L4 by maintaining energy reserves closer to  $E_{th}$  (the peak of the food assimilation trajectory); moreover, since both  $c_s$  and  $s$  are less than one, a larger fraction of the heightened food assimilated will be devoted to structural growth, as the proportion of food devoted to energy storage decreases.

Figures 4-11 and 4-12 show the impact of parameter variation on absolute energy reserves ( $E$ ) and percent energy reserves ( $E/W$ ). Although neither was experimentally evaluated, the former is directly linked to commitment to pupation (Gilpin and McClelland, 1979; Chambers and Klowden, 1990; Telang et al., 2007), whereas the latter is a likely indicator of the ability of the larva to withstand a reduction in resources at a particular stage of development. Metabolic parameters (red shades) clearly have little effect on energy reserves or percent energy reserves in non-starvation conditions (Figures 4-11 and 4-12). In contrast, increased maximum growth efficiency generates clear tradeoffs for larvae between the quantity and proportion of energy reserves. After 1-3 days feeding  $g$  has the strongest *positive* effect of all parameters on absolute energy reserves (Figure 4-11), but due to a stronger impact on weight, has paradoxically the strongest *negative* impact on proportion energy reserves (Figure 4-12). This indicates that

temperature driven increases in maximum growth efficiency (Figure 4-7, Table 4-2) will likely decrease time to maturation, but may reduce the ability of larvae to withstand food limited conditions. Extremely low values of  $g$  have highly irregular impacts on percent energy reserves (Figure 4-12) due to non-linear and differential impacts on weight (Figure 4-9) and total energy reserves (Figure 4-11).

In the vicinity of 28°C estimates, increasing  $g$  has a similar effect on total energy reserves as increasing  $c_s$  after 1-day feeding (Figure 4-11A), but clearly has a larger capacity to increase energy reserves after 2 days feeding and beyond (Figure 4-10B-D). The reason for this pattern is that  $g$  has a strong positive effect on food assimilated whereas  $c_s$  and  $s$  decrease food assimilated in the latter portion of development. Figures 4-11 and 4-12 also exhibit a change in the sensitivity of percent energy reserves to the coefficient ( $c_s$ ) vis-à-vis exponent ( $s$ ) of energy storage as development progresses. After one day feeding  $c_s$  improves both total and percent energy reserves, whereas  $s$  has little effect (Figures 4-11A and 4-12A). After two days feeding and beyond  $c_s$  and  $s$  have relatively similar effects on percent energy reserves in the vicinity of their 28°C values (Figures 4-11B-D and 4-12B-D).

Together with the estimated temperature dependence of the parameters (Figure 4-7), these results indicate that temperature-induced increases in development rate and metabolism have energetic costs that may influence larval capacity to mature in food-limited environments, assuming that the parameters do not vary with food conditions. By increasing energy storage with heightened temperature, larvae may energetically compensate for reduced energy reserves (due to an increased  $g$ ) and starvation resistance (due to an increased  $c_m$ ), but at the expense of final size, as predicted by the TSR. Such compensatory energy storage may occur either at the initial or final stages of larval development, through  $c_s$  or  $s$ , respectively.

#### 4.2.4.2 Sensitivity of observed starvation resistance to the efficiencies of growth and energy storage

Figures 4-9 to 4-12 show that in the model system, in the face of increasing maximum growth efficiency ( $g$ ) (Figure 4-7), attaining smaller final size at higher temperatures requires heightened energy storage. Our next step was to evaluate the sensitivity of the observed starvation resistance to simultaneous changes in growth and energy storage. We explored the joint parameter space of maximum growth efficiency ( $g$ ) and energy storage efficiency ( $c_s$ ) that reproduced observed mean starvation survival in each feeding-temperature regime. For this analysis groups of 4000 larvae were simulated across the parameter space of  $c_s$  and  $g$  (step of 0.15) including the ML estimates of each at all six temperatures. We employed the stochastic model with individual variations in initial lipids and final weight as previously described, in which the exponent of energy storage ( $s$ ) was individually fit to match asymptotic weight for each  $c_s$ - $g$  combination. As previously described  $c_m$  was chosen to match mean starvation resistance in unfed larvae at each temperature, given the value of  $m$  (see below).

The curves in Figure 4-13A-F depict the line bisection between the areas of the  $c_s$ - $g$  space that generated a mean starvation survival greater and less than the observed mean. For example, at 26°C (Figure 4-13D), mean starvation resistance of 4-day fed larvae (yellow curve) was reproducible within an extremely narrow range of  $g$  (~0.4) but through nearly the entire  $c_s$  range. Survival of 2 and 3-day fed larvae (green and red curves in Figure 4-13D) shows a larger interdependence and clear negative relationship between  $c_s$  and  $g$ , indicating a compensatory effect of the efficiency of growth vis-à-vis energy storage during the middle stages of development. Survival of 1-day fed larvae (blue curve in Figure 4-13D) is reproducible only when  $c_s$  is the vicinity of 0.13, whereas it can be reproduced across a wide range of  $g$  values. The energy storage exponent,  $s$ , clearly impacts the interdependency of the data on  $c_s$  and  $g$ . When

the system is insensitive to either of these parameters (at the beginning of development for  $g$  and the end of development for  $c_s$ ) they are both associated with  $s$ , although the association is much greater with  $c_s$  than  $g$  (i.e. in Figure 4-13D the small black numbers decline more rapidly going down the yellow curve than from right to left on the blue curve). This indicates that the insensitivity of the system to either parameter, particularly  $c_s$ , is due to the potential for compensatory effects in the exponent  $s$ . These effects of feeding age on the differential sensitivities of the system to  $g$  and  $c_s$  hold at all temperatures (Figures 4-13A to 4-13F).

At each temperature  $m$  was set at a value in which the curves converge at a single parameter region (see legend in Figure 4-13). The convergence regions do not correspond exactly to ML estimates for each parameter (Figure 4-7), because the curves depict the region where simulated and observed *mean* survival coincide, whereas ML parameter estimates were based on the probability that simulated larvae reproduced ML estimates of  $\lambda_{T,A}$  and  $k_{T,A}$ , based on the observed data. Discrepancies between model fits to the mean survival and the Weibull distribution may be caused by skewness and/or outliers in the observed or simulated data, which are accentuated by the increasing death hazard ( $k > 1$ ) over time in foodless conditions. Differences in the distribution of randomly assigned  $W_{L,t}$ , affecting the fit value of  $s$ , may have also contributed to discrepancies.

Nonetheless, the 1-day fed curves hover at a value relatively similar to the temperature-specific ML estimate for  $c_s$ , whereas the vertical (final day feeding) curves persist at values similar to the ML estimates for  $g$ . The increase in the level of  $c_s$  for survival of 1-day fed larvae (blue line) is particularly noteworthy at 28 and 30°C. These results are consistent with Figure 4-10, indicating that energy storage through the coefficient  $c_s$  impacts starvation resistance at the

beginning of larval development, whereas the exponent  $s$  can improve starvation resistance at the end of development.

Figure 4-13 also suggests that at higher temperatures there is a heightened sensitivity to maximum growth efficiency at earlier feeding ages. At 28 and 30°C, the slope of the 1-day (blue) shows a slight increase (negative) as compared to the lower temperatures and that of the 2-day fed curves (green) show a marked negative increase (Figures 4-13E and 4-13F). The slope of 2-day fed larvae at 30°C, in particular, is comparable to that of 3-day fed larva at 24 (Figure 4-13C) and 26°C (Figure 4-13D). This indicates the potential for increased interdependency of energy storage and growth efficiency in early instars at 28 and 30°C. Taken together with the experimentally observed increase in starvation resistance in the 1-day fed vis-à-vis unfed groups at 28 and 30°C (Experiment D, Figure 4-4), and the negative effect of heightened growth efficiency on percent energy reserves in the model (Figure 4-12), these results suggest the sensitivity of larval development to changes in specific parameters may vary with age; in particular, we suggest that above 26°C, larvae are compelled to store energy in earlier, as opposed to exclusively in later developmental stages; this may be a compensatory mechanism for high growth efficiency.

#### **4.2.5 Model Predictions**

##### **4.2.5.1 Weight, stored energy and reserve proportion as a function of temperature in unlimited food conditions**

Using parameter estimates of  $g$ ,  $m$ ,  $c_s$  and  $c_m$  (Figure 4-7) we projected accumulated energy stores and weight across all 6 temperatures. As previously described, energy storage was a stochastic process and  $E_o$  and  $W_{L4}$  were randomly assigned to each simulated larva, with the latter used to individually [re]fit  $s$ . Mean  $s$  among temperatures corresponded well to the values in Figure 4-7 (20°C:  $0.73 \pm 0.12$ , 22°C:  $0.83 \pm 0.13$ , 24°C:  $0.95 \pm 0.15$ , 26°C:  $1.0 \pm 0.14$ , 28°C:

$0.81 \pm 0.11$ ,  $30^\circ\text{C}$ :  $0.79 \pm 0.12$ ), as expected since all of the other parameters were identical. Figure 4-14 shows means and standard deviations of weight and energy reserves through the course of development as obtained from a simulation of 4000 larvae after 1-5 days of feeding on excess food. With warming temperature, energy reserves rise slower than weight in the early instars (Figure 4-14A and 4-14B), approximately proportional to weight in the middle of development (Figure 4-14C), and faster than weight in later instars (Figures 4-14D and 4-14E). Thus, the model predicts that the observed TSR in Experiment C (Figure 4-3) was generated due to the negative feedback of rising energy reserves on feeding rate in the last larval instar, in which most growth occurs (Gilpin and McClelland, 1979; Nishiura et al., 2007; Telang et al., 2007). In the latter feeding days, growth rate slows substantially in the higher temperatures, generating the predicted leveling off of the temperature-weight curve in 4-14D-E.

Concurrent with this pattern is a changing temperature dependency across development. In early stage larvae both weight and energy reserves increase at a higher rate in the warmer temperatures from  $26$ - $30^\circ\text{C}$ , but as development progresses the slope of the temperature curves is higher from  $20$ - $26^\circ\text{C}$ . This causes the gradual switch in the concavity of energy reserve and weight curves as development progresses (Figures 4-14A to 4-14E). The sharp increase in energy reserves from  $26$ - $30^\circ\text{C}$  after 1-day feeding (Figures 4-14A and 4-14B) parallels ML estimates for  $c_s$  (Figure 4-7A) and the heightened sensitivity of energy reserves to  $c_s$  at the beginning of development (Figure 4-11C). In contrast the heightened slope of weight and energy reserves from  $20$ - $26^\circ\text{C}$  in later developmental stages (Figures 4-14D and 4-14E) is likely a result of the increased storage exponent  $s$  at these temperatures (Figure 4-7B).

Using the same simulation conditions, we predicted proportion energy stores ( $E/W$ ) across temperature and feeding ages. This simulation was carried out until  $A_{pup}$  at each

temperature in order to predict how  $E/W$  in newly pupated mosquitoes may vary with temperature. Figure 4-15 shows a U-shaped pattern across temperatures after 1-3 feeding days, with lower values at 24 and 26°C as compared to the warmer and colder extremes. This is consistent with the larger increase in weight than energy reserves from 20-26°C and compensation of energy reserves from 26-30°C (Figures 4-14A and 4-14B). Taken together with Figures 4-12 and 4-13, these results indicate that increasing maximum growth efficiency ( $g$ ) leads to lower percent energy reserves unless it is compensated for in the early stages of development through  $c_s$ . The U-shape emerges because  $c_s$  does not rise until 28°C. After 4-days feeding, however, 24 and 26°C have larger percent energy reserves than 20 and 22°C, presumably due to a higher energy storage exponent  $s$ . Also noteworthy in Figure 4-15 is that percent energy reserves at  $A_{pup}$  increase roughly monotonically with increased temperature. This is consistent with the larger impact of temperature on energy reserves than on weight in the latter feeding ages (Figure 4-14).

#### **4.2.5.2 Combined effects of temperature and food limitation on development rate and cumulative pupation**

Using ML parameter estimates a value for the threshold energy reserves required for pupation ( $E_{pup}$ ) was fit based on Experiment A, the proportion of larvae that pupated subsequent to transfer into distilled water in each temperature-feeding age. This fit was carried out using 100 simulations of 40 larvae each, using the parameter values shown in Figure 4-7, with stochastic energy storage and individual variation in  $E_o$  and  $W_{L4}$ ; thus,  $s$  was again [re]fit to individual larvae in each simulation, yielding very similar average  $s$  values as in Figure 4-7. We assumed that  $E_{pup}$  corresponded to the average predicted energy reserves in the lowest percentile of larvae that pupated upon transfer to distilled water (Figure 4-1) Potential sex ratio biases, arising because males mature before females, were addressed by adjusting  $E_{pup}$  to the mean energy

stores in the larva with the  $n^{\text{th}}$  highest energy level in the feeding treatment in which at least 70% of larvae pupated after starvation, where  $n$  is the number of larvae that pupated after transfer to distilled water. This corresponded to 4 days feeding at 28 and 30°C, 5 days at 24 and 26°C, 6 days at 22°C and 7 days at 20°C. The fit value for  $E_{pup}$  was as follows for each temperature: 20°C:  $41.36 \pm 0.16$ , 22°C:  $51.04 \pm 0.19$ , 24°C:  $54.70 \pm 0.18$ , 26°C:  $53.82 \pm 0.26$ , 28°C:  $65.29 \pm 0.15$ , 30°C  $67.18 \pm 0.13$ . This pattern of increased  $E_{pup}$  at higher temperatures (notwithstanding the slightly lower value at 26 as compared to 24°C) is an expected outcome of our model fitting assumption of increased accumulation of energy reserves at higher temperatures, at a rate proportional to the data on commitment to pupation (Figure 4-1).

After fitting  $E_{pup}$  we simulated growth and pupation in 1000 larvae, varying  $F_{A,i,t}$  from 1 to 70  $\mu\text{g} / 6\text{h}$  and thereby including a range in which  $F_{A,i,t}$  fell below the right hand side of Equation 4-2 (i.e. no longer in excess). As with prior simulations in which  $F_{A,i,t}$  was equal to Equation 4-2, a constant amount of food was added at the beginning of each 6 h time step, all of which was presumed to be assimilated into biomass. This assumption presumes that the food available to a larva is equivalent to the food assimilated ( $F_{A,i,t}$ ).

Larvae were simulated in isolation in order to avoid making assumptions about resource competition. Stochastic energy storage and individual variation in  $E_o$  and  $W_{L4}$  were assumed as previously described. Values of  $E_o$ ,  $W_o$ ,  $\sigma$  and  $E_{th}$  were the same as those assumed in Table 1 and prior simulations. The only source of mortality assumed for each larva was depletion of energy reserves below zero. We varied the timing of onset of food limitation as follows: (1) immediately upon hatching, (2) after completing 2-days of feeding in excess ( $F_{A,i,t}$  equal to Equation 4-2), (3) early L4 (feeding in excess until median energy stores among the 1000 simulated larvae reached

$E_{th}$ ) and (4) middle L4 (feeding in excess until median energy stores matched the midpoint between  $E_{th}$  and  $E_{pup}$ ).

Figure 4-16 shows the effect of food level on the time required to attain 100% pupation allowing a maximum of 60 days to pupate. Figure 4-17 shows the cumulative pupation rate when the rate of food input is  $3\mu\text{g}/6\text{hrs}$ , which corresponded to the minimum food level in which pupation occurred within 40 days at all six temperatures, when food limitation onset immediately upon hatching. Both diagrams indicate that larvae in this model system are more sensitive to food limitation at 24 and 26°C, with consistently lower development rates in food limited scenarios. While this U-shaped relationship between temperature and development rate is more pronounced in early ontogeny, it is clearly maintained even when food limited conditions initiate when larvae are close to reaching  $E_{pup}$  (Figures 4-16D and 4-17D). The interaction between the effects of food and temperature on development rate are evident in the crossing of the curves in Figure 4-16. At higher food levels development rate increases monotonically with temperature with curves parallel to the x-axis (Figure 4-16), indicating that larvae reach  $E_{pup}$  before  $A_{pup}$ . At lower food levels, development rate is roughly similar at 20, 22, 28, and 30°C (Figures 4-16 and 4-17). Within these temperatures larval development rate is highest at 30°C, although the differences are slight and not consistent when food limitation begins at different developmental stages (Figures 4-16 and 4-17). These data indicate that a reduction in percent energy reserves at 24 and 26°C (Figure 4-15) may reduce the capacity of larvae to mature in food limited habitats. Together with Figures 4-13 and 4-14, these data suggest that if larvae do not energetically compensate for increased food assimilation early in development, rising temperatures enhances the effects of food limitation.

### 4.3 Discussion

Although heightened temperature increases the development rate of ectotherms, its ultimate effects on the abundance and distribution of species depend on how organisms compensate for increased energetic demands (Lafferty, 2009). Here we tested the hypothesis that the observation of reduced size at higher temperatures in ectotherms, the Temperature Size Rule, may be a consequence of this compensatory response. We modeled ectotherm development as a function of a negative feedback between growth and energy storage and compared it to laboratory experiments on the mosquito *Aedes aegypti*, carried out at 2°C interval between 20-30°C. Upon fitting the model to experimental observations of heightened development rate, lower final weight and reduced starvation resistance of unfed larvae with rising temperature, the model successfully reproduced independent data on starvation resistance through the course of development at all six temperatures. Sensitivity analyses on the key temperature dependent parameters indicated a simple, novel and potentially general, mechanistic explanation for temperature effects on ectotherm growth and development: that heightened temperature produces a net *increase* in the rate of accumulation of energy reserves, which reduces the rate of conversion of food into biomass in the final larval instar. Moreover, our findings indicate that the developmental stage at which compensatory energy storage occurs may qualitatively change with small temperature increments. These results suggest a potential interaction between temperature and the resources available to mosquito larvae that could cause local habitat features to modify the effects of temperature on the dynamics of mosquito production.

Most explanations of the TSR in ectotherms cite differential impacts of temperature on the rate or allometric scaling of growth and metabolism (Berrigan and Charnov, 1994; Perrin, 1995; Angilleta et al., 2004). Implicit in these explanations is the assumption that weight and energy stores are interchangeable and directly proportional to one another (Strong and Daborn,

1980; Angilleta and Dunham, 2003). Since these explanations assume that weight determines maturation rate and final size, tradeoffs between these two developmental outcomes generating the TSR can only arise through the differential impacts of temperature on the coefficients or exponents in the growth equation (Bertalanffy, 1960). However, at least in mosquitoes, such a formulation ignores evidence that implicates energy reserves, and not weight *per se* in the causal mechanism associated with pupation (Gilpin and McClelland, 1979; Chambers and Klowden, 1990; Telang et al., 2007). Here, we model independently the dynamics of energy storage and weight gain in developing *A. aegypti* larvae. Each of these processes were linked to each other through the assumption that storing energy favors increasing growth rate up to a period of exponential growth in the early fourth instar, after which further energy surplus reduces food conversion into biomass (Equation 2). In this model mosquitoes that store more energy commit to pupation sooner and additionally reduce food intake in the latter portion of the last larval instar. This mechanism reduces development time in larvae that store more energy, thereby shortening the interval to cessation of growth (ICG), the period between commitment to pupation and attainment of final weight (Davidowitz and Nijhout, 2004, Nishiura et al., 2007).

Besides the generality of the TSR itself, there are a number of pieces of information from empirical studies that support the basic premise of our model and the tradeoffs we attribute to rising temperature. First, despite the arbitrary assumption of 40% initial energy reserves, our results indicate that temperature induced depletion of energy reserves has little direct impact on observed body size variation, as evidenced by the low sensitivity of weight to variation in the metabolic coefficient ( $c_m$ ) and exponent ( $m$ ) (Figure 4-9). This is likely due to the low values of  $c_m$  relative to weight and is supported by Telang et al. (2007), who found that fourth instar *A. aegypti* did not have significantly reduced weight after starvation for 36 hours. Secondly, the

sigmoidal weight trajectory generated by our use of a Gaussian function to model growth rate with decreasing food assimilation in the ICG, is well supported in laboratory studies of *A. aegypti*, in addition to those presented here (Dye, 1984; Telang et al., 2007). Finally, in *A. aegypti* and in most ectotherms, growth efficiency tends to increase with temperature (Rashed and Mulla, 1990; Angilleta and Dunham, 2003), coinciding with our ML estimates that show a linear increase in maximum food assimilation efficiency ( $g$ ) from 20 to 30°C. Using our model, we demonstrate that increasing  $g$  is the key process that permits greater accumulation of energy reserves and thereby faster commitment to pupation at higher temperatures (Figures 4-1); however, this may generate a deficit in energy reserves as a percent of body weight in the absence of compensatory energy storage in the early instars (Figure 4-12). Moreover, the model predicts that in early development rising temperature increases weight gain more than energy reserves, whereas the reverse is true at later developmental stages (Figure 4-14). We suggest that our proposed mechanism of a negative feedback between energy storage and food assimilation in the last larval instar may provide a general explanation for the temperature-size rule in holometabolic ectotherms.

While asymptotic size decreases with temperature, asymptotic energy reserves as a proportion of body weight ( $E/W$ ) is predicted to increase with temperature (Figures 4-14 and 4-15). Sensitivity analyses in concert with temperature-specific parameter estimates indicated that while temperature-induced increases in growth efficiency ( $g$ ) reduce percent energy reserves (Figure 4-12) by increasing weight more than reserves, temperature effects on energy storage parameters ( $c_s$  and  $s$ ) more than compensate by reducing weight and increasing reserves (Figures 4-9 and 4-11). This “overcompensation” may allow *A. aegypti* larvae in higher temperatures to overcome the effects increased metabolic coefficient ( $c_m$ ) on faster depletion of energy reserves

and reduced starvation resistance (Figure 4-10). Thus, because food limitation (Arrivillaga and Barrera, 2004; Barrera et al., 2006b) and stochastic food entrance (Subra and Mouchet, 1984) are common regulators of urban *A. aegypti* population dynamics (Southwood et al., 1972), it may be that *A. aegypti* energetically overcompensates in order to reduce vulnerability to food scarcity. However, the high frequency of food limitation in *A. aegypti* habitats means that heightened energy reserves with temperature are unlikely to be observed in field *A. aegypti* populations. Moreover, increased temperature may increase expenditure of larval reserves in the pupal and/or adult stages, thereby providing another adaptive benefit of heightened percent reserves in larvae. We suggest that temperature-induced energetic compensation should be investigated in adult stages and in other mosquito species, particularly those that readily inhabit anthropogenic habitats with large variation in resource availability, such as *A. albopictus* or *Anopheles gambiae* spp.

Our data indicate that crossing a critical temperature may generate a qualitative change in the developmental stage in which compensatory energy storage occurs in *A. aegypti*. Sensitivity analyses show that while energy storage can occur through either the coefficient or the exponent of energy storage, increases in the coefficient  $c_s$  will favor energy reserves and starvation resistance in both the early and latter stages of development (Figures 4-10 to 4-12). In contrast, increased storage exponent  $s$  improves energy balance only after 2-days of optimal feeding (Figures 4-10 to 4-12). ML parameter estimates show an increasing  $s$  and relatively constant  $c_s$  from 20-26°C. In contrast  $c_s$  shows a sharp linear rise in the 26-30°C range, whereas  $s$  drops at 28 and 30°C to the levels of 22 and 20°C (Table 4-2, Figure 4-7). Moreover, the model fit to starvation resistance is highly sensitive to  $c_s$  at 1-day feeding, with little dependence on  $g$  or  $s$  (Figure 4-13). In the middle stages of development the impact of  $c_s$  on the model fit was highly

dependent on the values of  $g$  and  $s$  (Figure 4-13). Interestingly, at 30°C the dependency of observed starvation resistance of  $c_s$  on values of  $g$  arises in earlier feeding days (Figure 4-13F), suggesting a decreased window for compensatory energy storage at high temperature.

The model and ML estimates suggest that the experimentally observed increase in starvation resistance in the 1-day fed as compared to unfed groups at 28 and 30°C (Figure 4-4) was due to an increase in energy storage at the initial stages of development (Figure 4-13), in response to high growth efficiency (Figure 4-4). At 24 and 26°C, ML estimates (relative to 20 and 22°C) suggest that increased metabolism ( $c_m$ ) and growth efficiency ( $g$ ) without an early increase in energy storage ( $c_s$ ) generated the observed decline in starvation resistance after 1-day feeding in comparison to unfed larvae (Figure 4-4). This interpretation of ML estimates is also consistent with the reduced starvation resistance in the 2-day fed group at 24 and 26°C, in comparison to 22, 28 and 30°C (Figure 4-5). These results support the notion of a “norm of development” (Angilleta et al., 2004), whereby ectotherms modify developmental strategies in response to temperature variation. In the case of *A. aegypti*, we suggest the potential for a threshold temperature that generates a switch in energy compensation strategies from end of development to the beginning of development; in our laboratory system this occurred between 26 and 28°C. We speculate that such a response could involve an epigenetic trigger in early ontogeny, such that larvae initially raised at 28°C and subsequently transferred to 26°C would exhibit heightened starvation resistance compared to larvae raised continuously at 26°C.

In summary, the model suggests three different developmental patterns in our experimental temperature treatments: 20-22°C: low metabolic demand, low energy storage and increased growth towards the end of development; 24-26°C: increased food assimilation and metabolic demand early in ontogeny with compensatory energy storage late in ontogeny; 28-

30°C: high metabolism, food assimilation and energetic storage at the beginning of development. As a result of these patterns, percent energy reserves at the beginning and middle stages of development are predicted to be higher at 20, 22, 28 and 30°C as compared to 24 and 26°C (Figure 4-15). We show that in food limited environments this could translate into a U-shaped relationship between temperature and development rate. The model predicts a substantially lower capacity to mature in food limited environments at mid range temperatures (24-26°C in our experimental system) as compared to the colder and warmer extremes of the thermal optima of *A. aegypti* (Figures 4-16 and 4-17). Interestingly, while percent energy reserves at 24 and 26°C are predicted to recover after 3 and 4 days of optimal feeding (Figure 4-15), when food limitation onsets in beginning or middle of the last instar, it continues to have a larger impact on maturation time at these temperatures as compared to the colder temperatures (Figures 4-16C and D and 4-17C and D). This is likely due to an increased effect of metabolic parameters on starvation resistance when food is limited (Figure 4-10), since larvae must expend energy reserves in order to maintain basic functions (Eq. 4-5).

Understanding how disease vectors respond to temperature changes is essential for predicting their abundance and distribution in heterogeneous environments. Our results indicate that increased temperature may cause mosquitoes to assimilate more resources in early ontogeny and less in late ontogeny. Furthermore, we show that amidst increased metabolic and food assimilation rates, temperature-induced changes in the timing of compensatory energy storage can generate an interaction between the effects of food availability and temperature on *A. aegypti* development time (Figures 4-16 and 4-17). This interaction calls into question the fundamental assumption of a monotonic relationship between the rate of emergence and warming temperature in the 20-30°C range (Sharpe and DeMichele, 1977; Schoolfield et al., 1981; Rueda et al., 1990)

and the temperature-driven predictions of *A. aegypti* production models that are based on this assumption (Focks et al., 1993; Jetten and Focks, 1997; Kearney et al., 2009; Magori et al., 2009; Williams et al., 2009). Testing our predicted resource-temperature interaction in experimental vessels in which water temperature, larval density and food input are simultaneously varied can lead to the development of improved models in order to assess vector populations in heterogeneous environments.

The prediction of an interactive effect of temperature and resources on mosquito development rate suggests that temperature-driven predictions of the rate of adult emergence in *A. aegypti* should be habitat specific, and at larger spatial scales may depend on the container landscape of a particular area. Moreover, the effects of food limitation, via larval competition and/or limited opportunity for nutrient entrance, may vary among altitudes, latitudes and seasons. For example, we can speculate that in a region where temperature fluctuates between 25 and 28°C, seasonal variation in *A. aegypti* production may be much larger in a high housing density urban community with intra-domiciliary *A. aegypti* container habitats exposure to few nutrient sources, as compared to a peri-urban community in which largely outdoor vessels receive increased nutrient input. Given that in many countries mosquitoes readily transmit disease amidst temperatures that vary on the order of 4-6 degrees in the 20-30°C range, understanding how these results apply to other mosquito species and strains of *A. aegypti* will be an important step in determining the long term impact of climate change and variation on mosquito borne disease.

Table 4-1. Parameters of model and used to describe experimental data

| Equation               | Symbol          | Description   | Estimation and key assumptions   |
|------------------------|-----------------|---|--|
| 4-2, 4-3,<br>4-4, 4-5  | $W_o$           | Initial weight of newly hatched larva   | Temperature independent, assumed constant among larvae at experimentally measured mean value, 4.1 $\mu\text{g}$ .  |
| Used for model fitting | $W_{L4}$        | Weight of late L4/early pupae   | Measured individually in larvae/pupae (n=30) at each temperature; randomly assigned to each simulated larva using a distribution based on measurements.  |
| 4-5                    | $E_o$           | Initial mean stored energy of newly hatched larva                                     | Temperature independent; mean set arbitrarily at 1.5 $\mu\text{g}$ or 40% $W_o$ ; individual variation randomly generated using a distribution derived from variation in starvation resistance of unfed larvae |
| 4-2                    | $E_{th}$        | Amount of stored energy that triggers exponential food assimilation (beginning of L4) | Temperature independent; set arbitrarily at 15 $\mu\text{g}$ (10 times $E_o$ ).  |
| 4-2                    | $\sigma$        | Spread of gaussian feeding rate   | Temperature independent, set at 23 $\mu\text{g}$ ; value roughly consistent with 28°C weight trajectory given $E_{th}$ assumption (above) over a range of values for $g$ , $m$ and $c_s$                       |
| 4-2, 4-4,<br>4-5       | $c_m$           | energy depletion coefficient  | Chosen to match mean survival of unfed larvae at each temperature, using equation 2b, given the values of $E_o$ and $m$  |
| 4-4                    | $s$             | energy storage exponent   | Chosen to match asymptotic weight assigned to each larva, given the value of $c_s$ , $m$ and $g$ .   |
| 4-2, 4-4,<br>4-5       | $m$             | energy metabolism exponent  | ML estimate based on starvation resistance and commitment to pupation at each temperature. 28°C ML estimate also based on weight trajectory from 1-3 days feeding.   |
| 4-4                    | $c_s$           | energy storage coefficient  | Same as $m$  |
| 4-2                    | $g$             | Maximum growth efficiency   | Same as $m$  |
| 4-1, 4-7               | $\lambda_{T,A}$ | Weibull scale parameter for starvation resistance                                     | ML fit to experimental data from each temperature and feeding starvation treatment, based on Weibull hazard function   |

Table 4-1. Continued

| Equation                          | Symbol    | Description   | Estimation and key assumptions   |
|-----------------------------------|-----------|---|--|
| 4-1                               | $k_{T,A}$ | Weibull shape parameter for starvation survival         | Same as $\lambda_{T,A}$  |
| Used in food limitation scenarios | $E_{pup}$ | Amount of stored energy reserves that triggers pupation | Chosen to match energy reserves of minimum percentile that pupated after transferal to distilled water at each temperature for feeding regimes in which at least 70% of starved larvae pupated |
| 4-2, 4-3, 4-4, 4-5, 4-7           | $A$       | Feeding age   | Days of continuous larval feeding upon hatching  |
| Used in food limitation scenarios | $A_{pup}$ | Median developmental time required to pupate            | Days of feeding after which at least 50% of larvae pupated   |

Table 4-2. Joint narrow range ML parameter space for maximum growth efficiency (g), coefficient of energy storage ( $c_s$ ) and exponent of metabolism ( $m$ )

| Temperature (°C) | Maximum growth efficiency (g) | Coefficient of energy storage ( $c_s$ ) | Metabolism exponent ( $m$ ) |
|------------------|-------------------------------|---|-----------------------------|
| 20               | 0.2-0.35                      | 0.1-0.25                                | 0.3-0.5                     |
| 22               | 0.25-0.4                      | 0.1-0.25                                | 0.45-0.6                    |
| 24               | 0.3-0.5                       | 0.1-0.25                                | 0.5-0.65                    |
| 26               | 0.35-0.5                      | 0.1-0.25                                | 0.45-0.6                    |
| 28               | 0.45-0.6                      | 0.2-0.35                                | 0.45-0.6                    |
| 30               | 0.5-0.65                      | 0.25-0.4                                | 0.45-0.6                    |

Note: Each parameter was varied at 0.05 intervals through the 0-1 range. Ranges represent the two values for each parameter that generated the highest likelihood, with 0.05 added to each end of the range. At 28°C ML fits are based on the summation of the likelihood of the observed hazard function for starvation resistance ( $LL_{H,T}$ ) and weight trajectory ( $LL_{W,28}$ ). At all other temperatures, estimates are based solely on  $LL_{H,T}$  with no other constraints outside the fits of  $s$  and  $c_m$  to  $W_{L4}$  and starvation resistance of unfed larvae, respectively. Mean  $E_o=1.5\mu\text{g}$ ,  $\sigma=23\mu\text{g}$ ,  $E_{th}=15\mu\text{g}$  (Table 4-1).

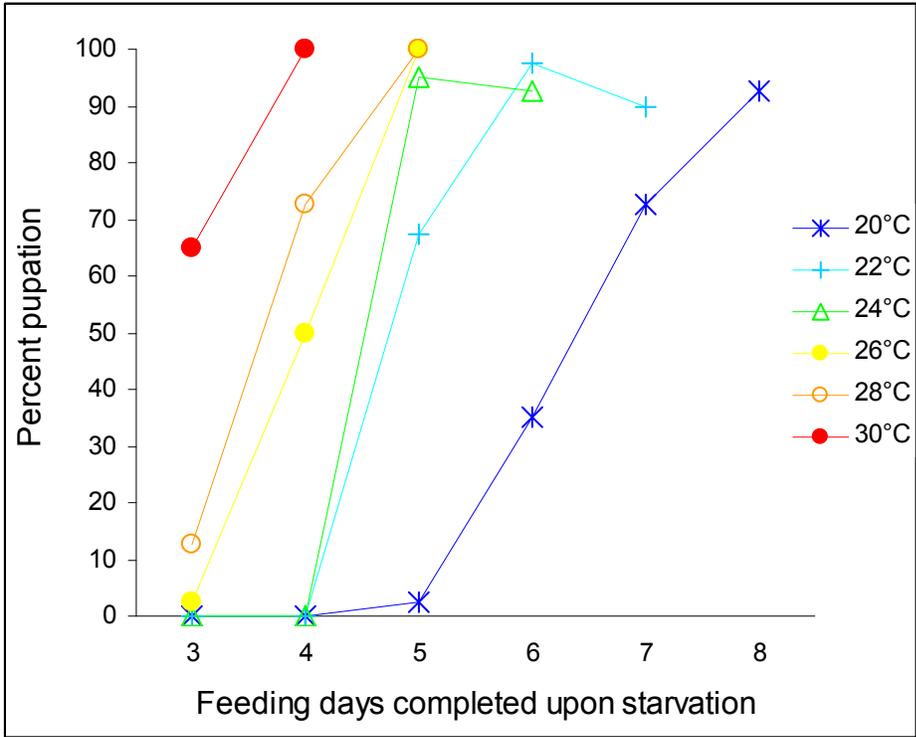


Figure 4-1. Minimum feeding days required to pupate upon transfer to distilled water across temperatures. Larvae experience starvation mortality in those treatments with less than 100% pupation. Curves at each temperature terminate on the day in which pupae first appear before transfer to starving conditions.

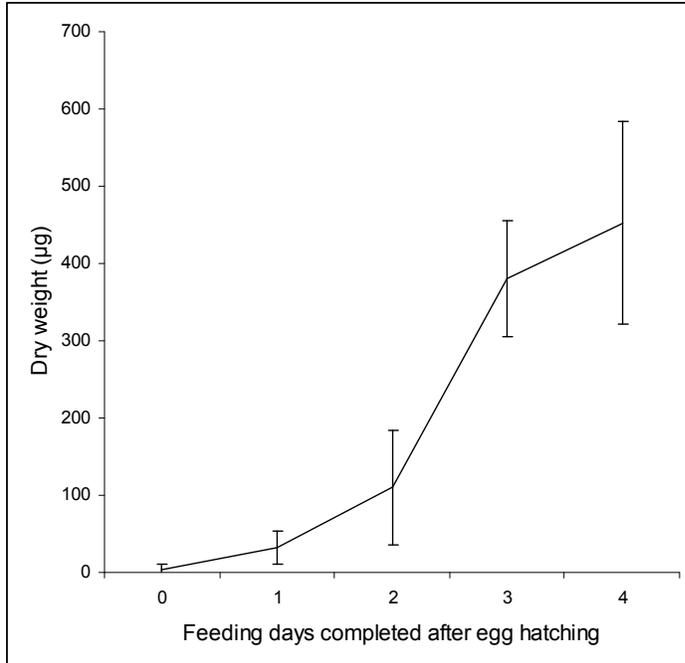


Figure 4-2. Weight trajectory at 28°C. Error bars show standard error of dry weight across groups of larvae multiplied by group size (n) in each feeding group as follows: newly hatched (n=10), 1-day fed (n=5) and 2-day fed (n=4) and 3-day fed (n=2); 4-day fed larvae were weighed individually (n=30).

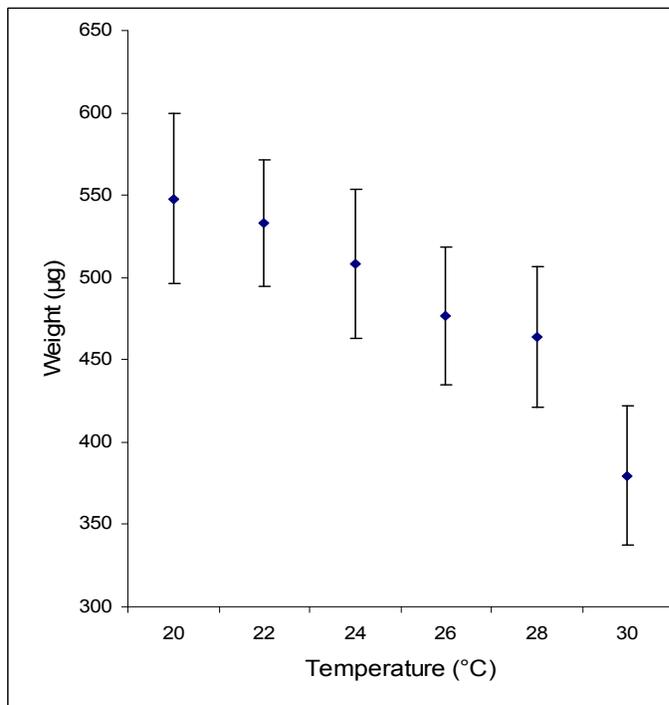


Figure 4-3. Mean  $W_{L4}$  ( $\pm$  95% CI) among temperatures. Measured after 4, 5, 6, 7 and 8 days feeding at 30, 28, 26, 24, 22, 20°C respectively.

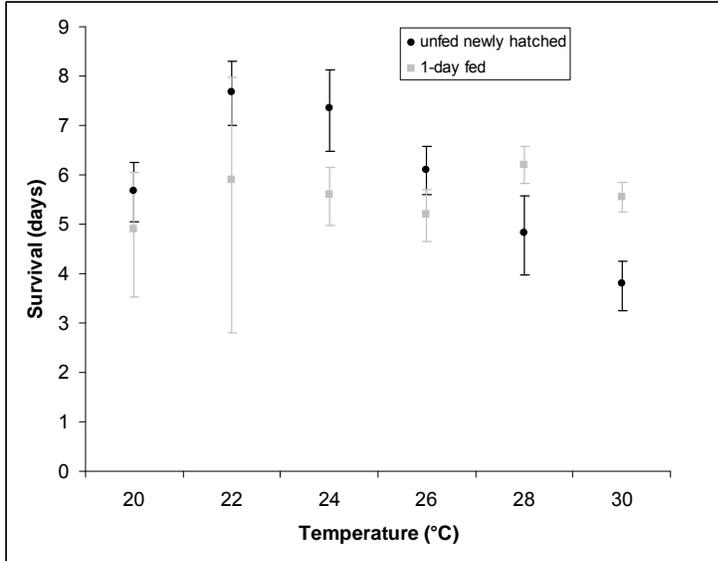


Figure 4-4. Starvation survival in unfed newly larvae and in 1-day fed larvae. Starvation survival as measured by the scale parameter ( $\lambda$ ) of the Weibull distribution ( $\pm 95\%CI$ ) in unfed newly larvae (in black) and in 1-day fed larvae (in grey).

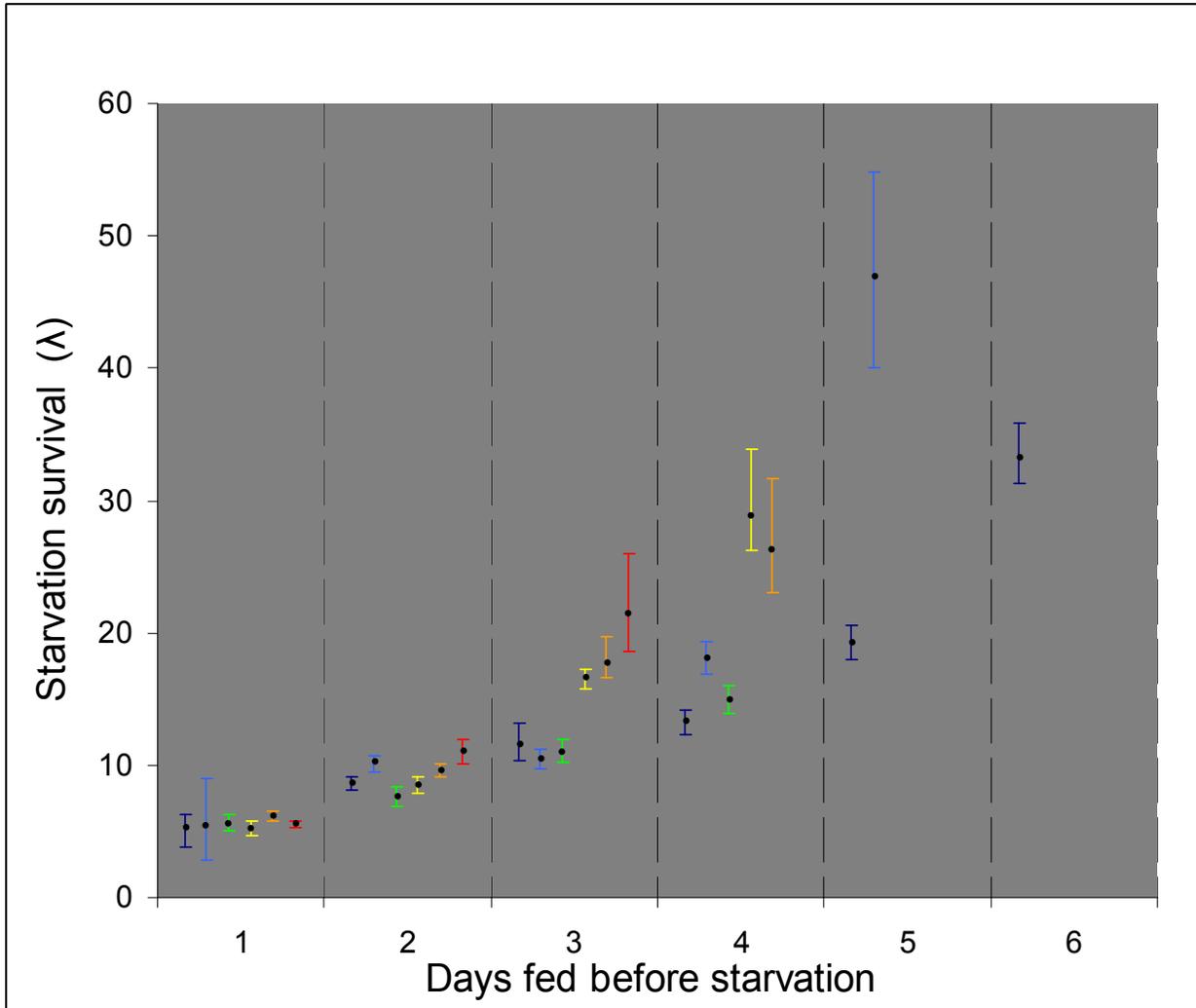


Figure 4-5. Starvation survival, as measured by the Weibull scale parameter ( $\lambda$ ) of starved *A. aegypti* among temperature and feeding treatments. Color coding is as follows: dark blue: 20°C, light blue: 22°C, green: 24°C, yellow: 26°C, orange: 28°C, red: 30°C. Data from one day fed larvae are repeated from Figure 4-4. In all treatments at least 10 larvae experienced starvation mortality without pupating. Maximum feeding time complying with this criterion was 3 days at 30°C, 4 days at 28, 26, and 24°C, 5 days at 22°C and 6 days at 20°C.

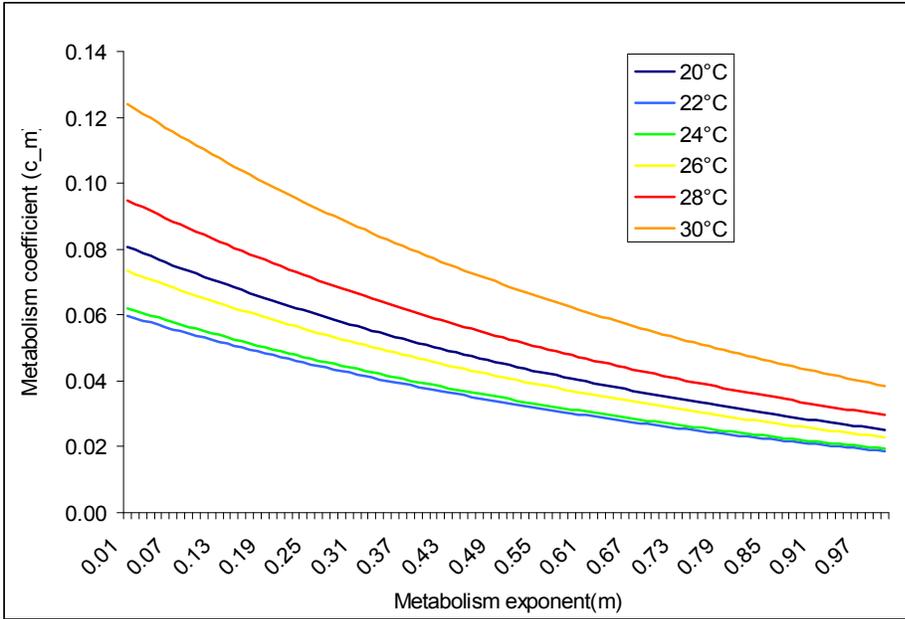


Figure 4-6. Joint space of coefficient ( $c_m$ ) and exponent ( $m$ ) of metabolism that reproduces mean starvation resistance of unfed larvae from 20 to 30°C.

A

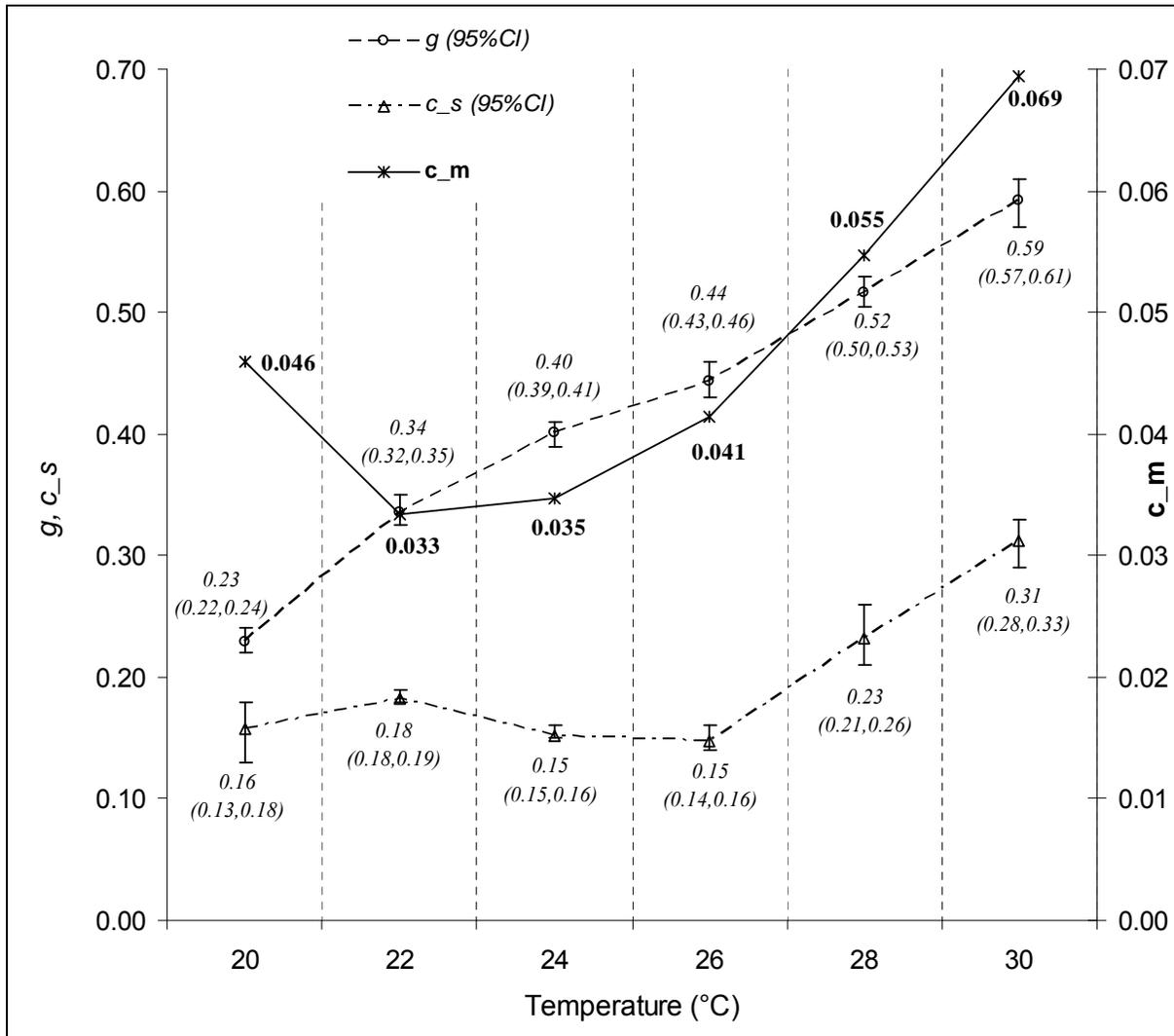


Figure 4-7. Estimates of temperature dependent parameters based on Experiments A-D: A) Coefficients of growth (maximum growth efficiency,  $g$ ), energy storage ( $c_s$ ) and metabolism ( $c_m$ ). B) Exponents of metabolism ( $m$ ) and energy storage ( $s$ ). 95% CIs of parameters estimated through ML analysis ( $g$ ,  $c_s$ , and  $m$  as described in Table 4-2 are based on 100 repetitions of the ML analysis (see text) for each temperature. Mean  $E_o = 1.5 \mu\text{g}$ ,  $\sigma = 23 \mu\text{g}$ ,  $E_{th} = 15 \mu\text{g}$  (Table 4-1). The energy storage exponent ( $s$ ) is chosen to match the  $W_{L4}$  assigned to each larva; mean and SD among larvae are given in the figure. The coefficient of metabolism ( $c_m$ ) is fit to mean starvation resistance of unfed larvae given the ML value of  $m$ ; it is assigned the same value to each larva.

B

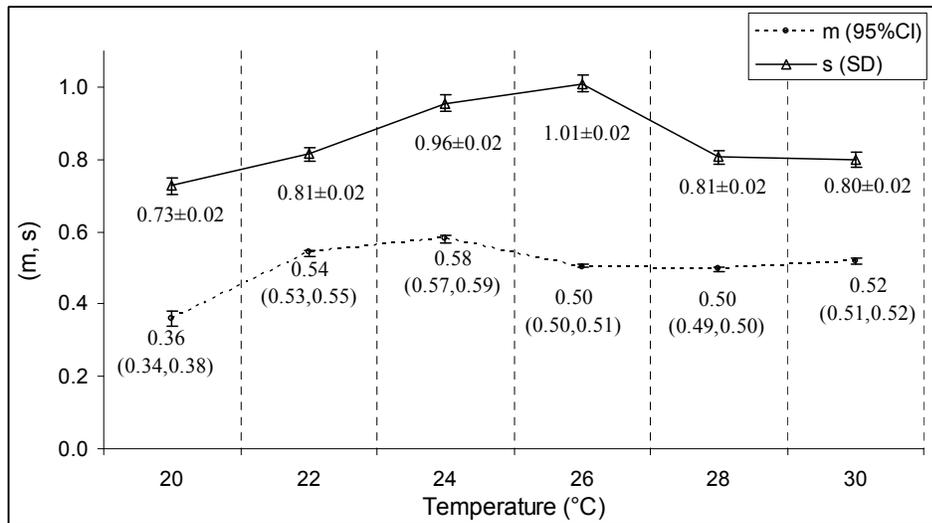


Figure 4-7. Continued

A

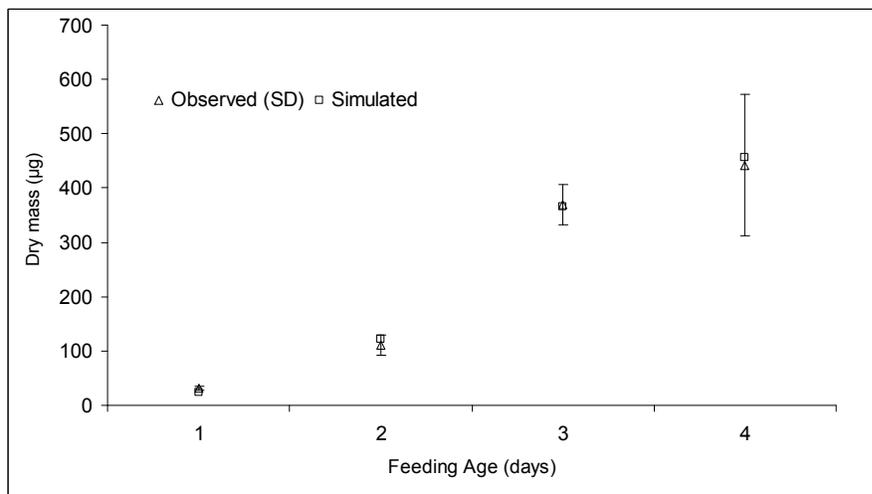


Figure 4-8. Maximum likelihood model fits to observed data. A) Model fit to observed weight trajectory after 1-3 days feeding at 28°C. Simulated 4-day fed weight is fit to the observed distribution among larvae (see text) Error bars show standard error of dry weight across groups of larvae for 0 (n=10), 1 (n=5) and 2 (n=4) and 3 (n=2) days fed groups. For 4-day fed larvae bars are standard errors across individuals (n=30). B) Maximum likelihood estimates of mean starvation survival across feeding age at 20, 22, 24, 26, 28 and 30°C. Triangles and circles are observed and simulated means, respectively. Pupation is not accounted for in model fitting; for treatments in which larvae pupated after transfer to starvation, the corresponding number of simulated larvae with the highest energy reserves after feeding are removed prior to calculation of means. Dotted error bars are observed SDs, solid error bars are SDs among 4000 simulated larvae.

B

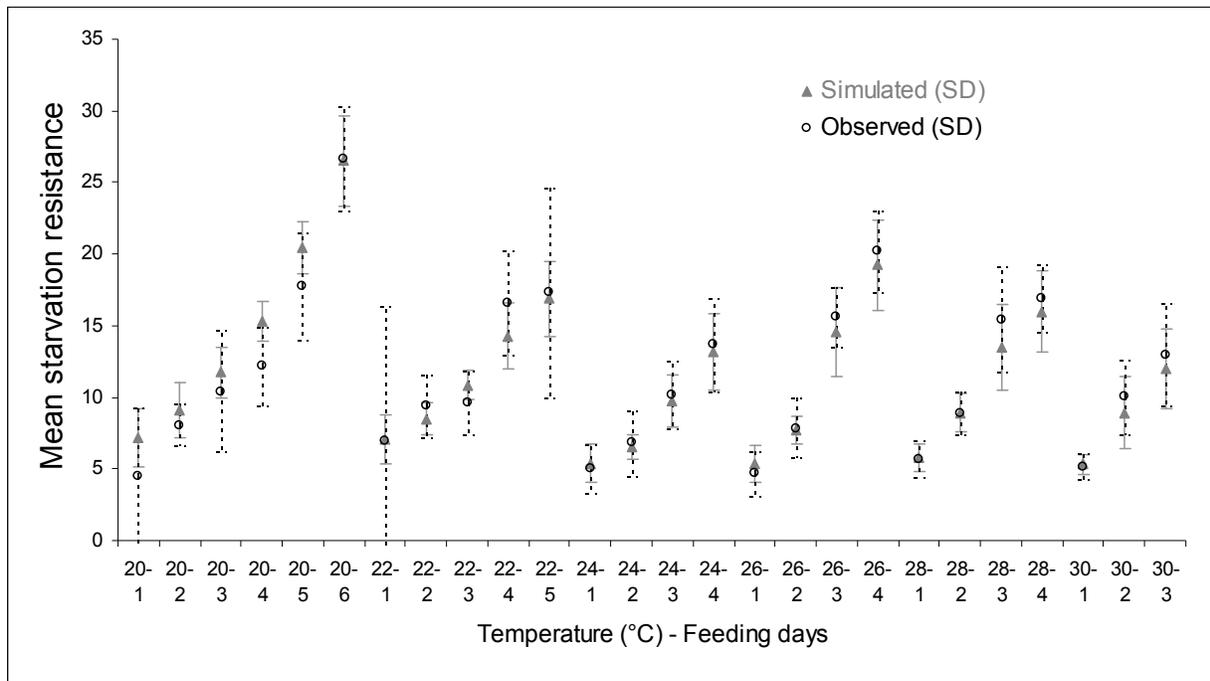


Figure 4-8. Continued

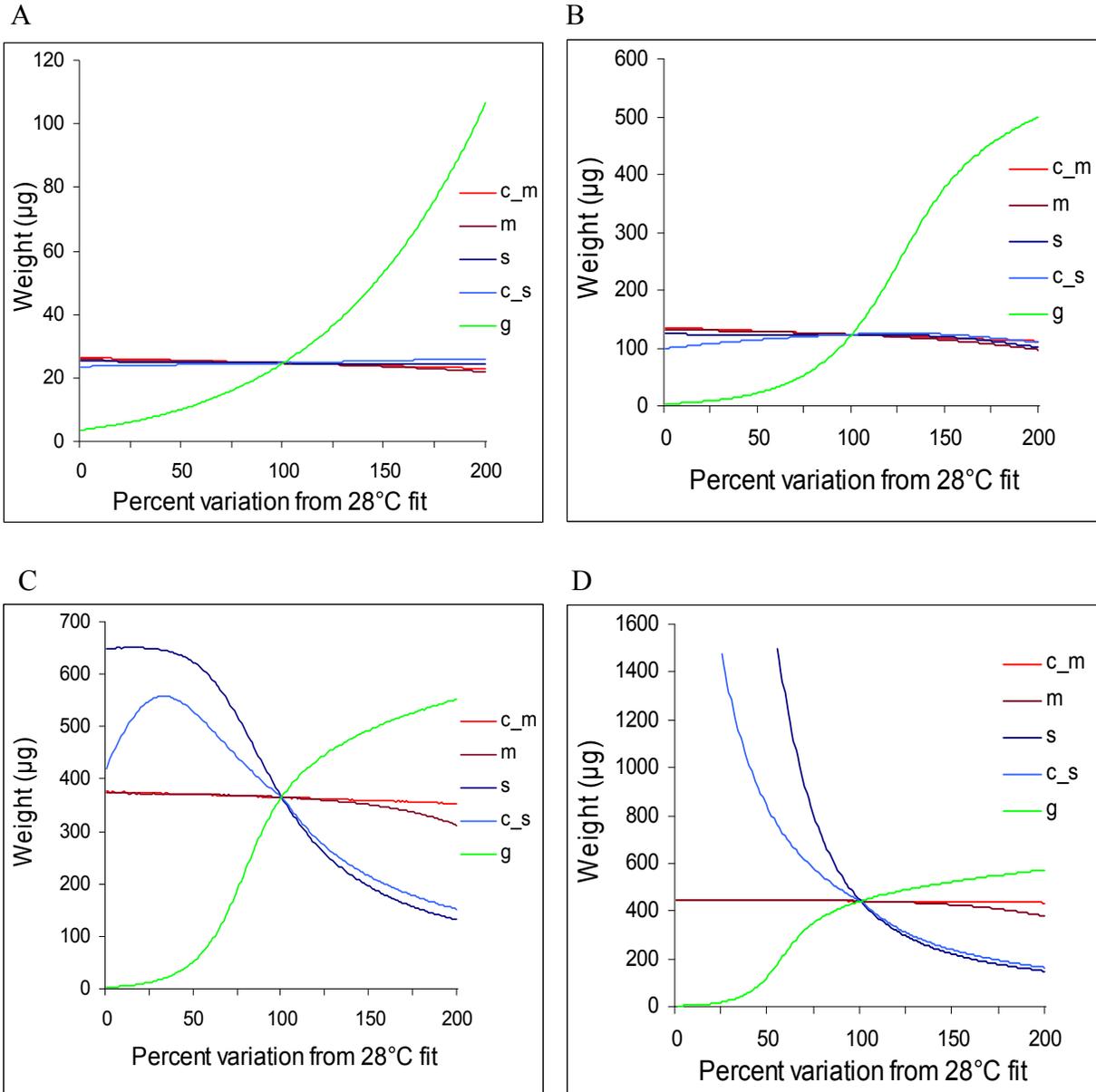


Figure 4-9. Sensitivity of weight after 1 to 4 days feeding to variation in model parameters with respect to 28°C ML fit. A) 1-day fed, B) 2-day fed, C) 3-day fed, D) 4-day fed. Maximum 1500µg weight allowed. Shades of red are energy metabolism parameters ( $c_m$  and  $m$ ), shades of blue ( $c_s$  and  $s$ ) are energy storage parameters and green is maximum growth efficiency ( $g$ ). Each temperature dependent parameter is varied individually, maintaining all others at mean value given in Figure 4-7.  $E_o=1.5\mu\text{g}$ ,  $\sigma=23\mu\text{g}$ ,  $E_{th}=15\mu\text{g}$  (Table 1). No individual variation in  $E_o$ ,  $s$  or stochastic energy storage; one larva simulated.

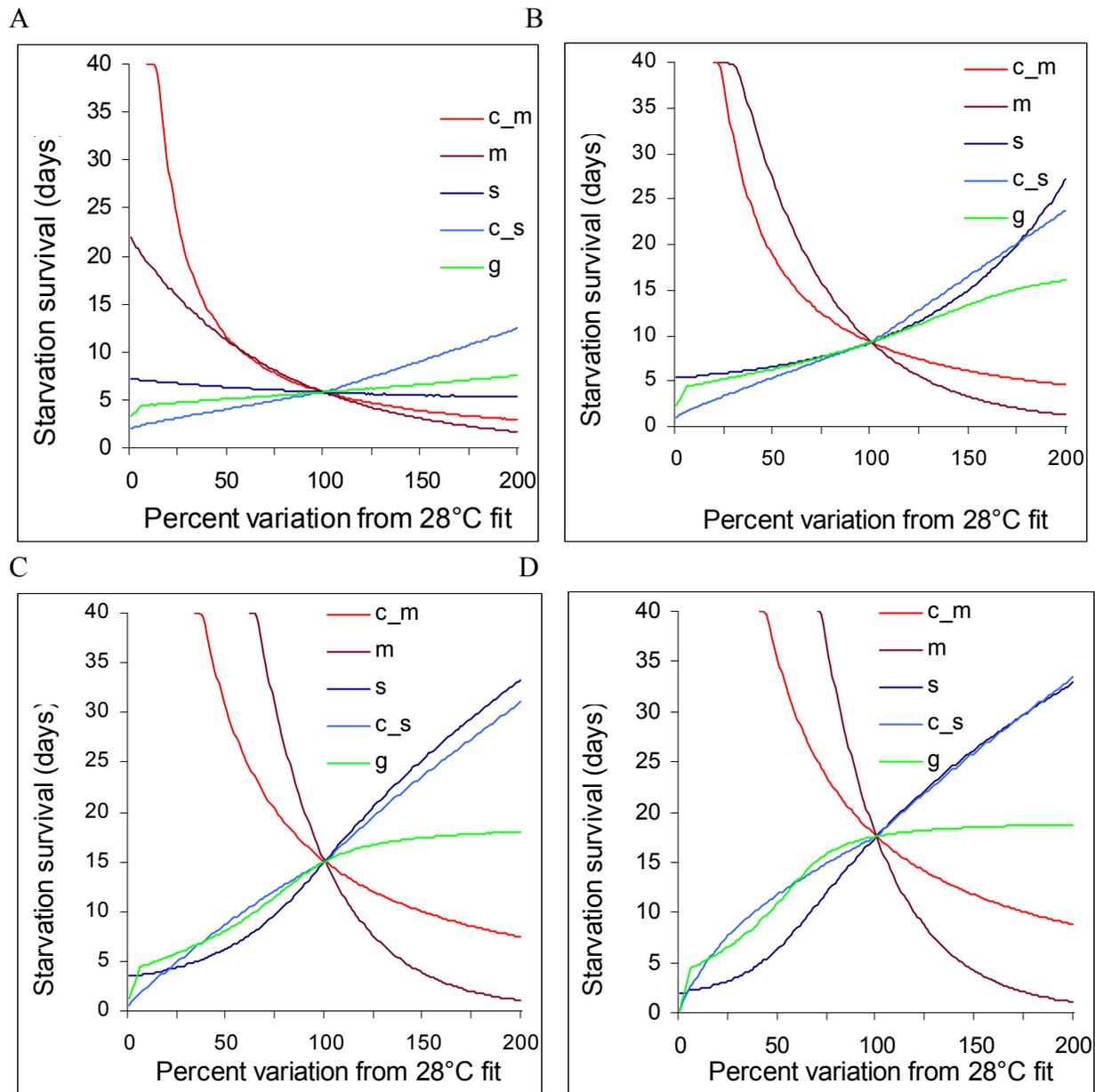


Figure 4-10. Sensitivity of starvation survival after 1 to 4 days feeding to variation in model parameters with respect to 28°C fit. A) 1-day fed, B) 2-day fed, C) 3-day fed, D) 4-day fed. Maximum 40 days survival allowed. Shades of red are energy metabolism parameters ( $c_m$  and  $m$ ), shades of blue ( $c_s$  and  $s$ ) are energy storage parameters and green is maximum growth efficiency ( $g$ ). All parameter values and simulation conditions are identical to Figure 4-9.

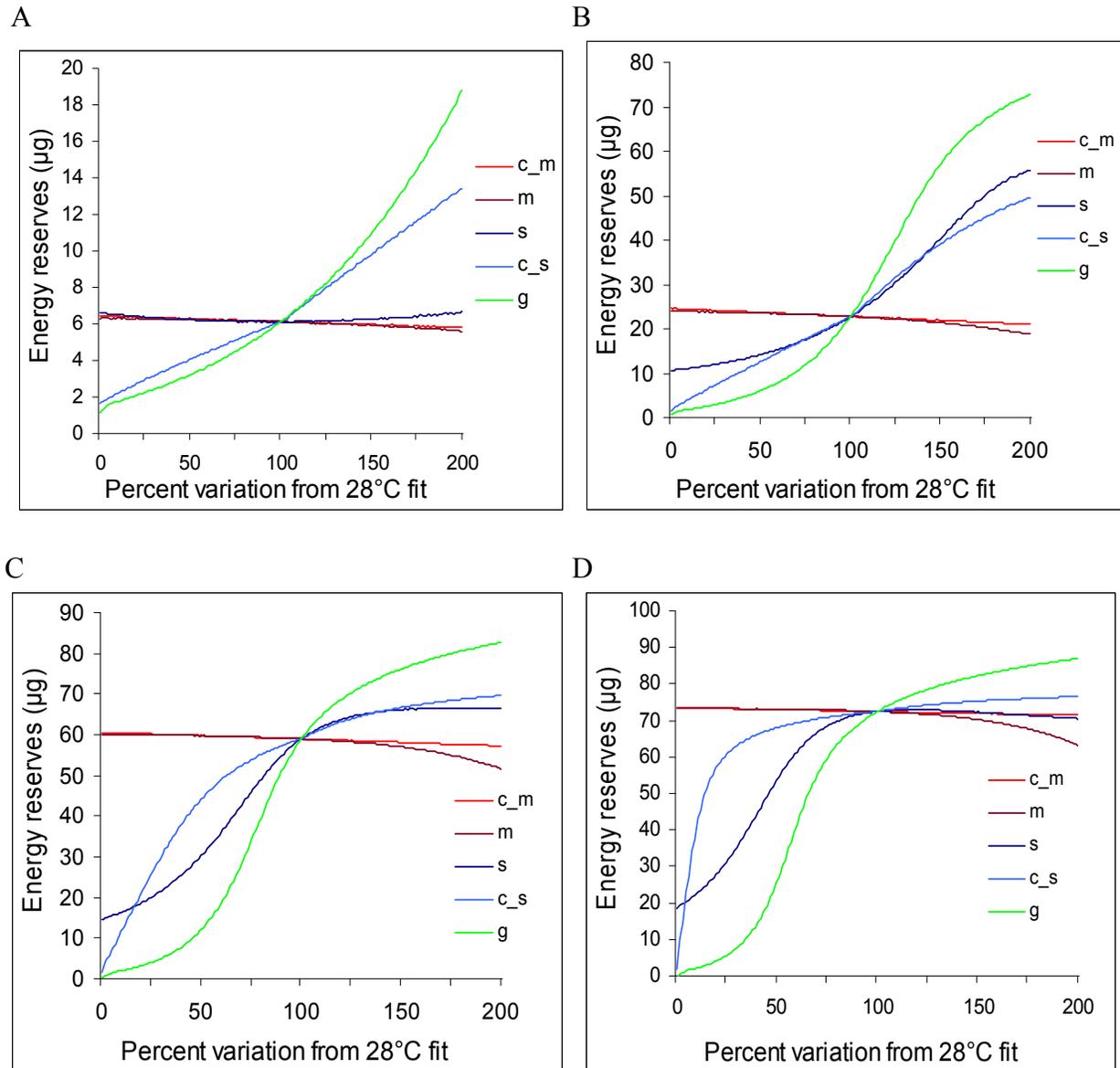


Figure 4-11. Sensitivity of energy reserves (E) after 1 to 4 days feeding to variation in model parameters with respect to 28°C fit. A) 1-day fed, B) 2-day fed, C) 3-day fed, D) 4-day fed. Shades of red are energy metabolism parameters ( $c_m$  and  $m$ ), shades of blue ( $c_s$  and  $s$ ) are energy storage parameters and green is maximum growth efficiency ( $g$ ). All parameter values and simulation conditions are identical to Figure 4-9.

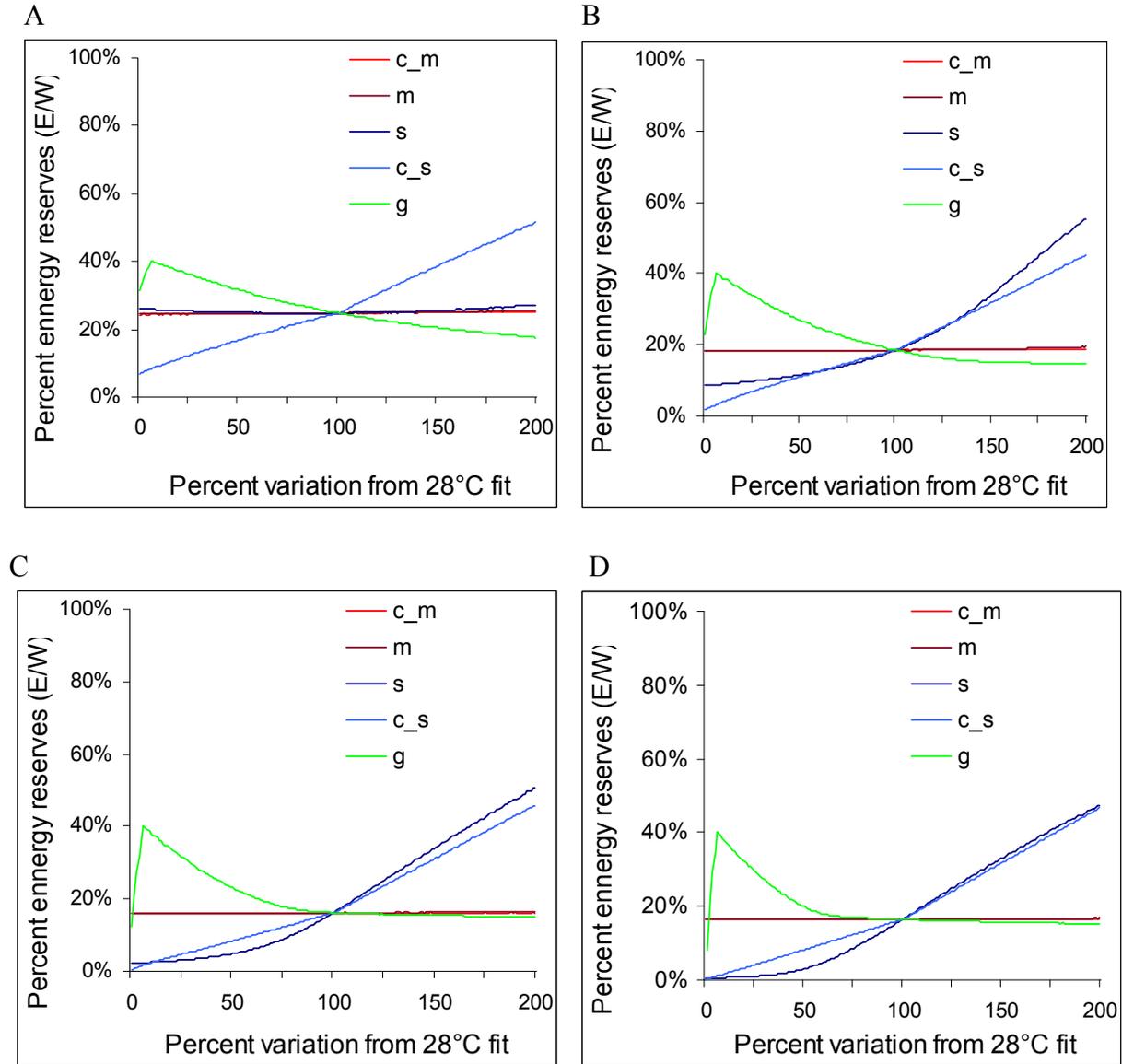


Figure 4-12. Sensitivity of proportion of energy reserves (E/W) after 1 to 4 days feeding to variation in model parameters with respect to 28°C fit. A) 1-day fed, B) 2-day fed, C) 3-day fed, D) 4-day fed. Shades of red are energy metabolism parameters ( $c_m$  and  $m$ ), shades of blue ( $c_s$  and  $s$ ) are energy storage parameters and green is maximum growth efficiency ( $g$ ); All parameter values and simulation conditions are identical to Figure 4-9.

A

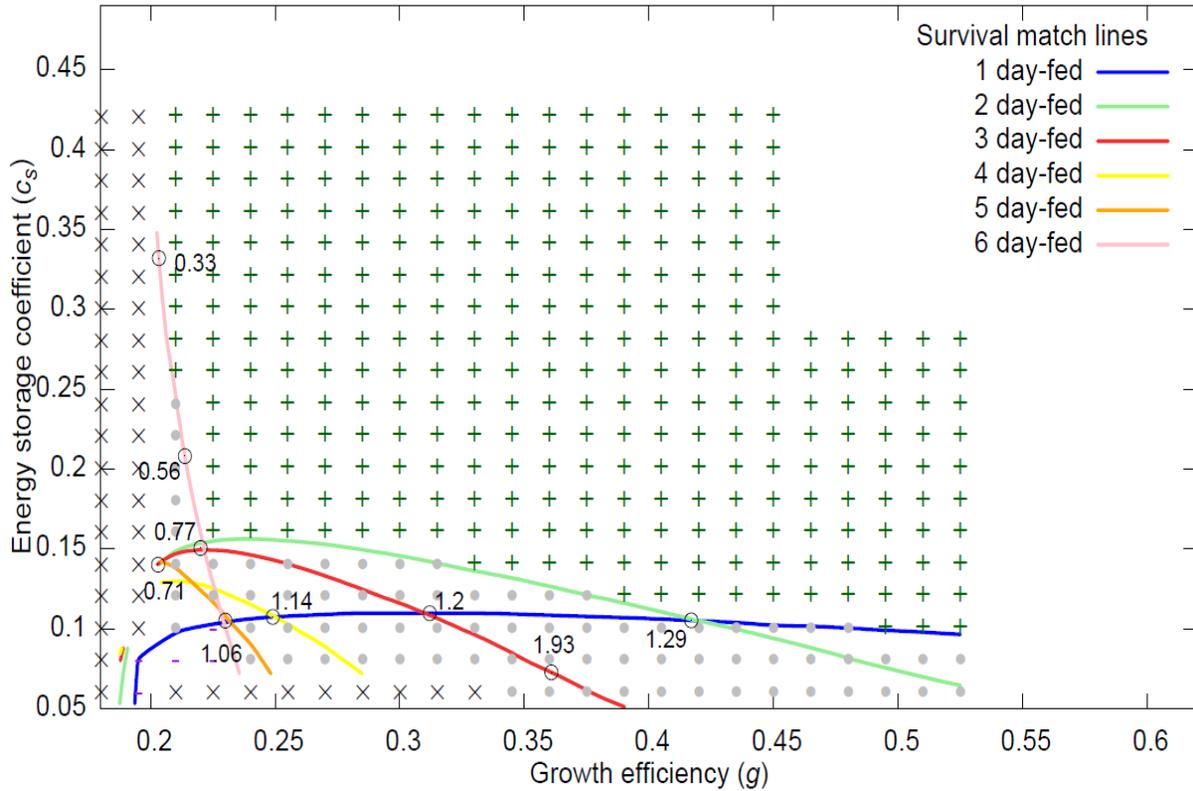
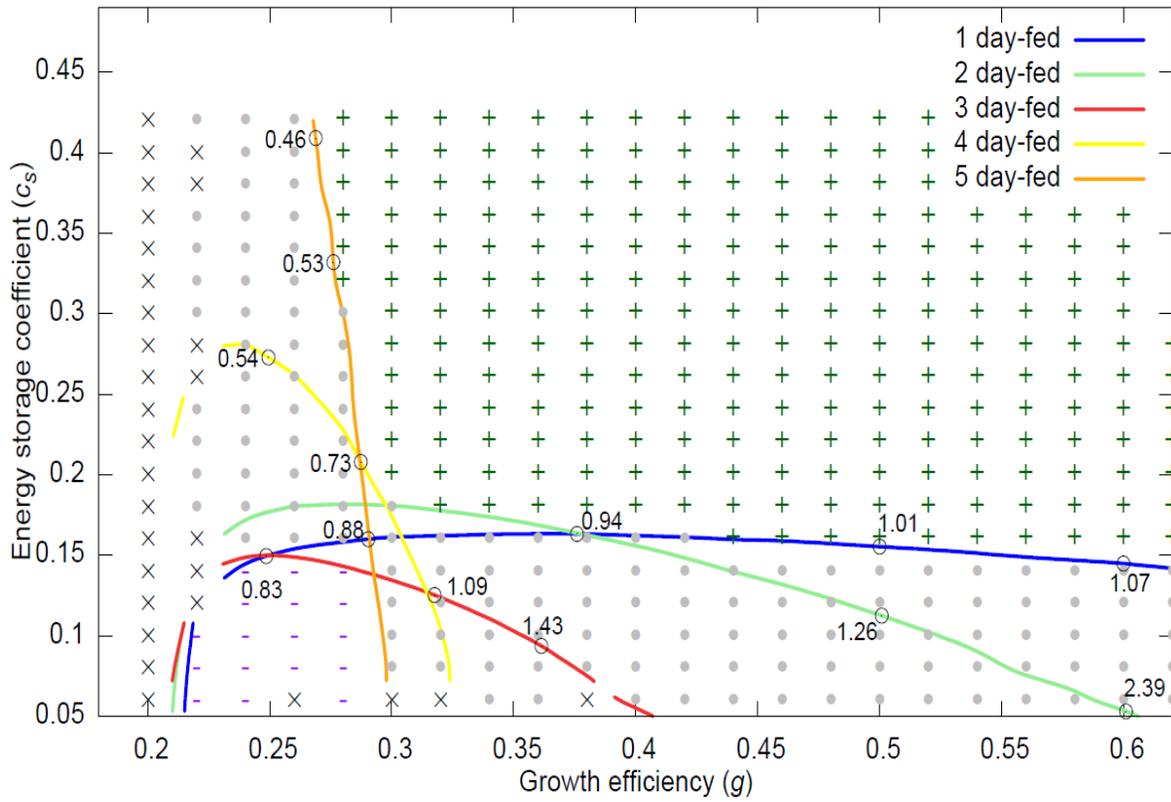


Figure 4-13. Profiles of simulated versus experimental survival for variation in growth efficiency ( $g$ ) and energy allocation ( $c_s$ ), by temperature and feeding age: A) 20°C. B) 22°C. C) 24°C. D) 26°C. E) 28°C. F) 30°C. Each curve represents the joint  $g$  and  $c_s$  space in which the model reproduces observed starvation resistance, with feeding treatments color-coded. “+” indicates survival is greater than observed for all feeding ages, “-” indicates lower simulated survival at all feeding ages, “•” indicates higher survival at some feeding ages and lower at others, and “x” means that asymptotic weight could not be reproduced if the energy storage exponent  $s$  took on a positive value. Numbers adjacent to lines are the average fit value of the energy allocation exponent  $s$  for a particular parameter combination. For all temperatures  $E_o=1.5\mu\text{g}$ ,  $\sigma=23\mu\text{g}$ ,  $E_{th}=15\mu\text{g}$  (Table 4-1). Metabolism coefficient ( $c_m$ ) and exponent ( $m$ ) used for 20°C:  $c_m=0.062$ ,  $m=0.30$ ; 22°C:  $c_m=0.039$ ,  $m=0.47$ ; 24°C:  $c_m=0.04$ ,  $m=0.60$ ; 26°C:  $c_m=0.049$ ,  $m=0.46$ ; 28°C:  $c_m=0.060$ ,  $m=0.47$ ; 30°C:  $c_m=0.075$ ,  $m=0.47$ .

B



C

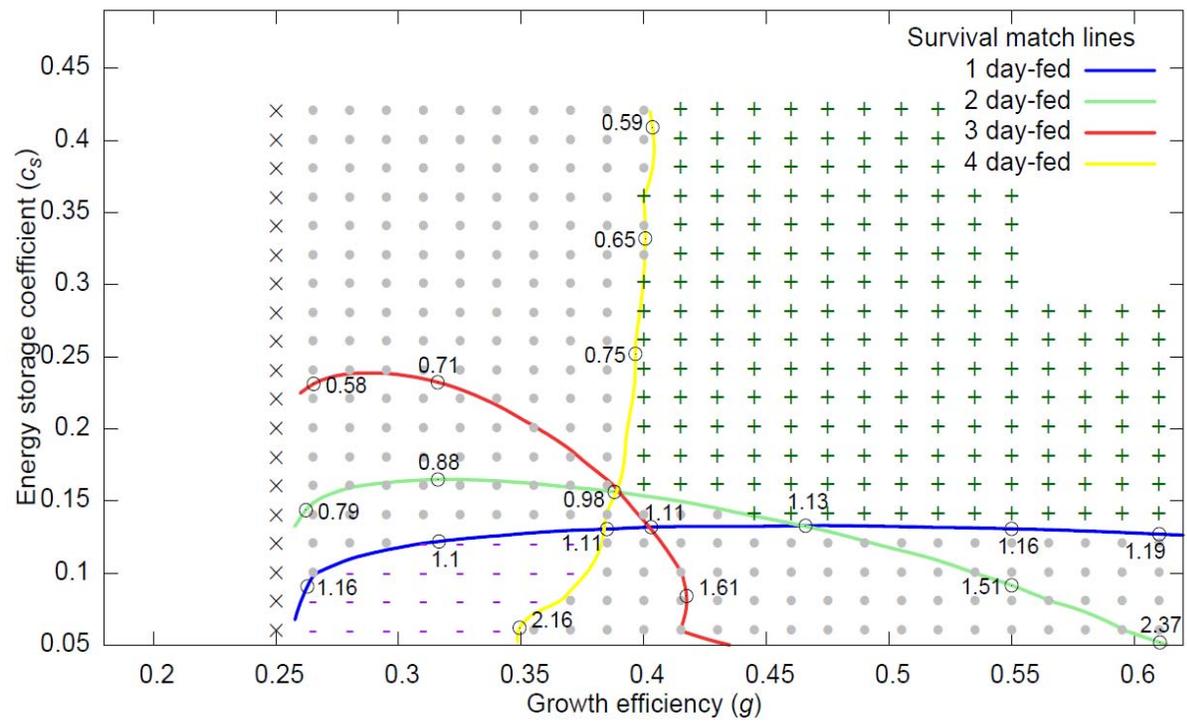
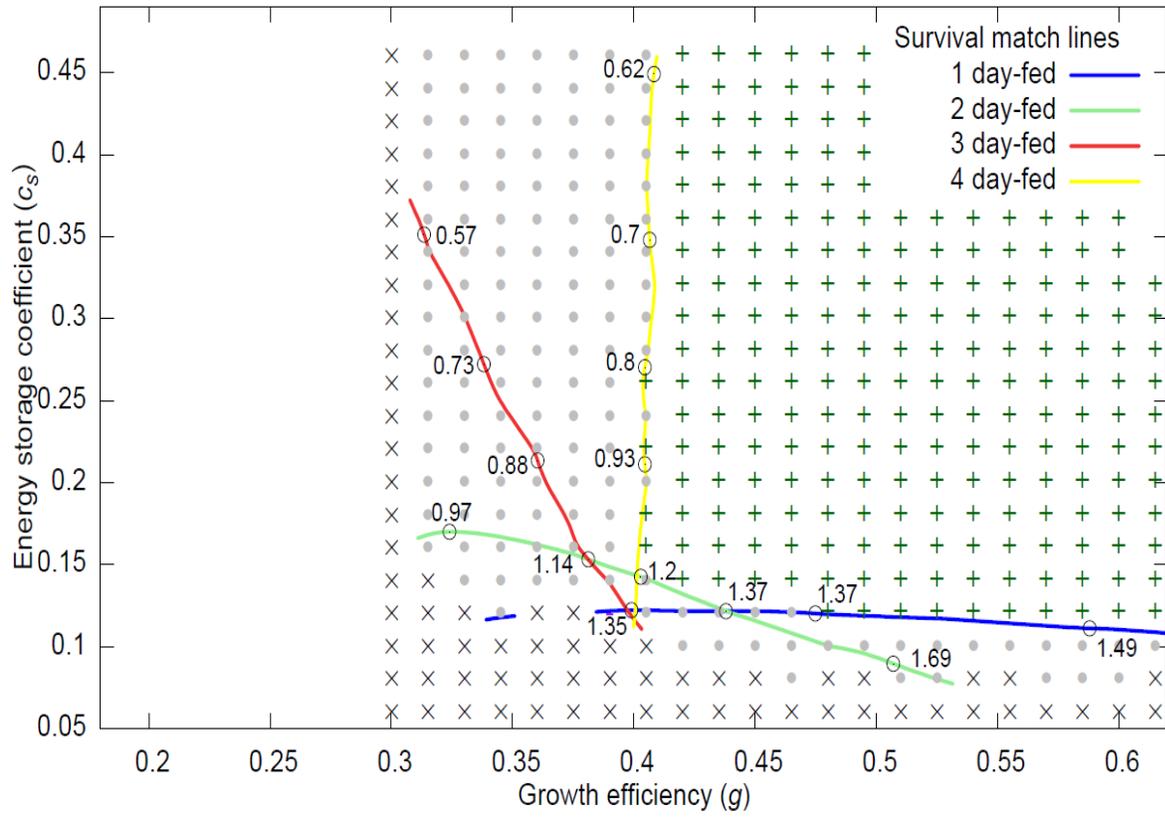


Figure 4-13. Continued

D



E

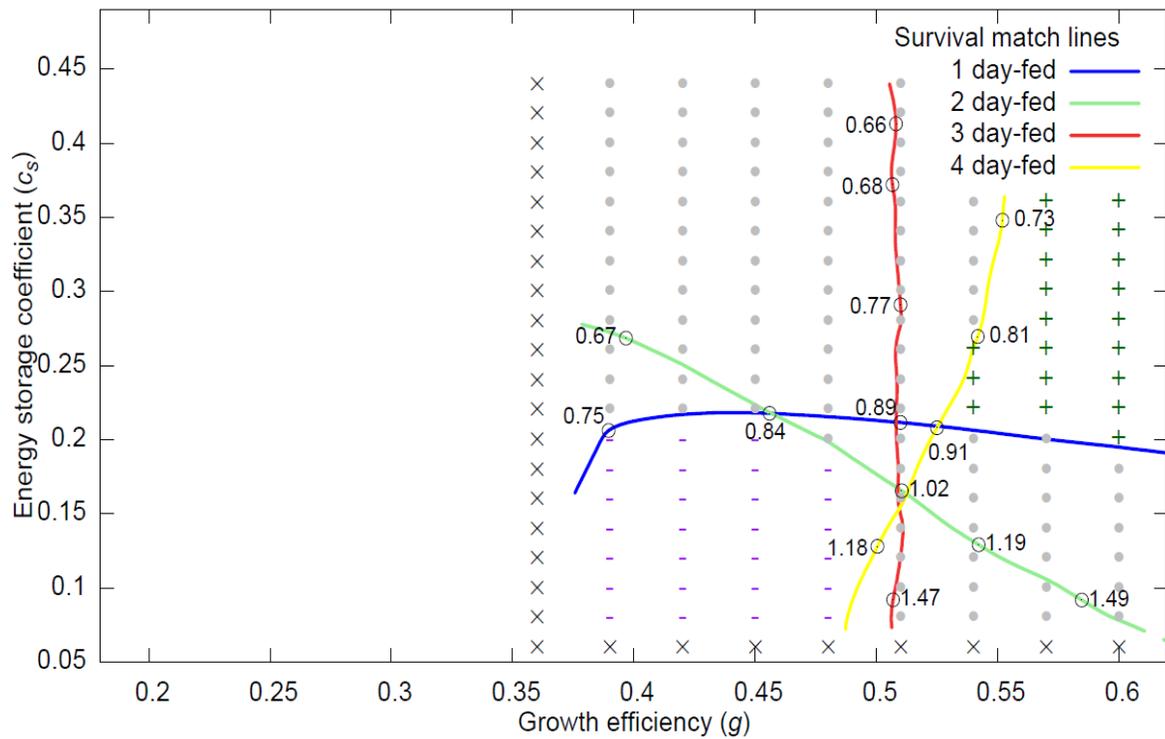


Figure 4-13. Continued

F

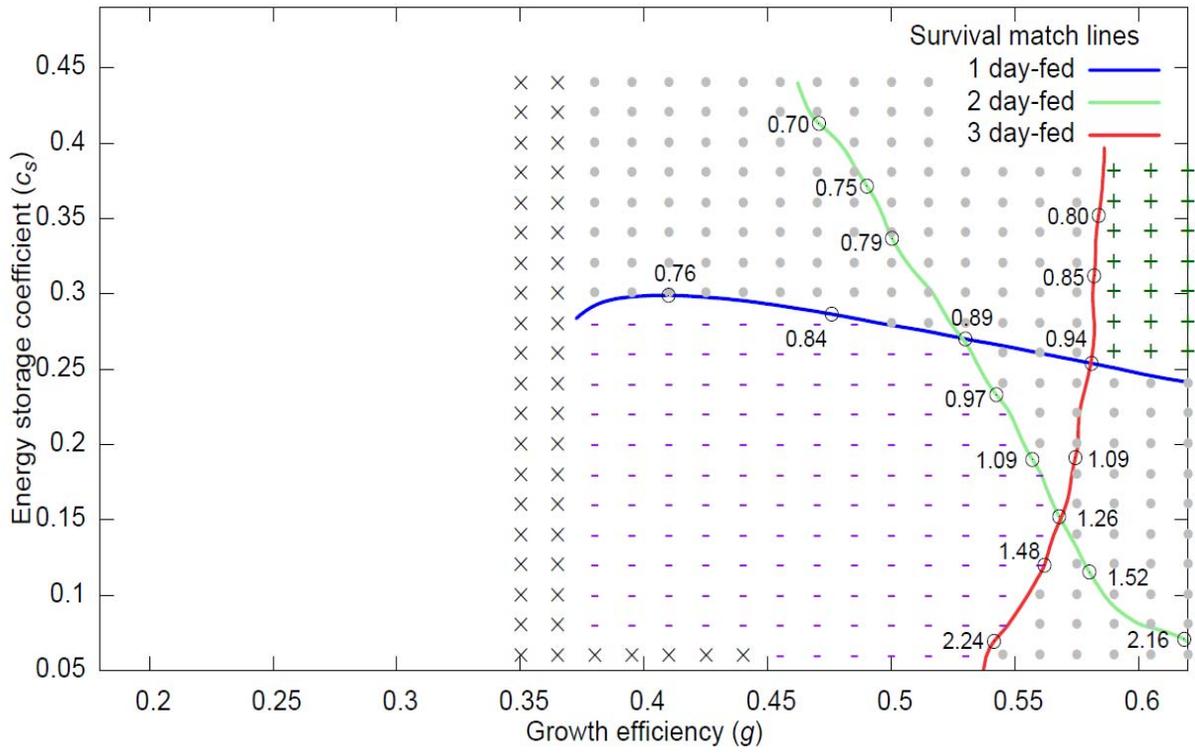


Figure 4-13. Continued

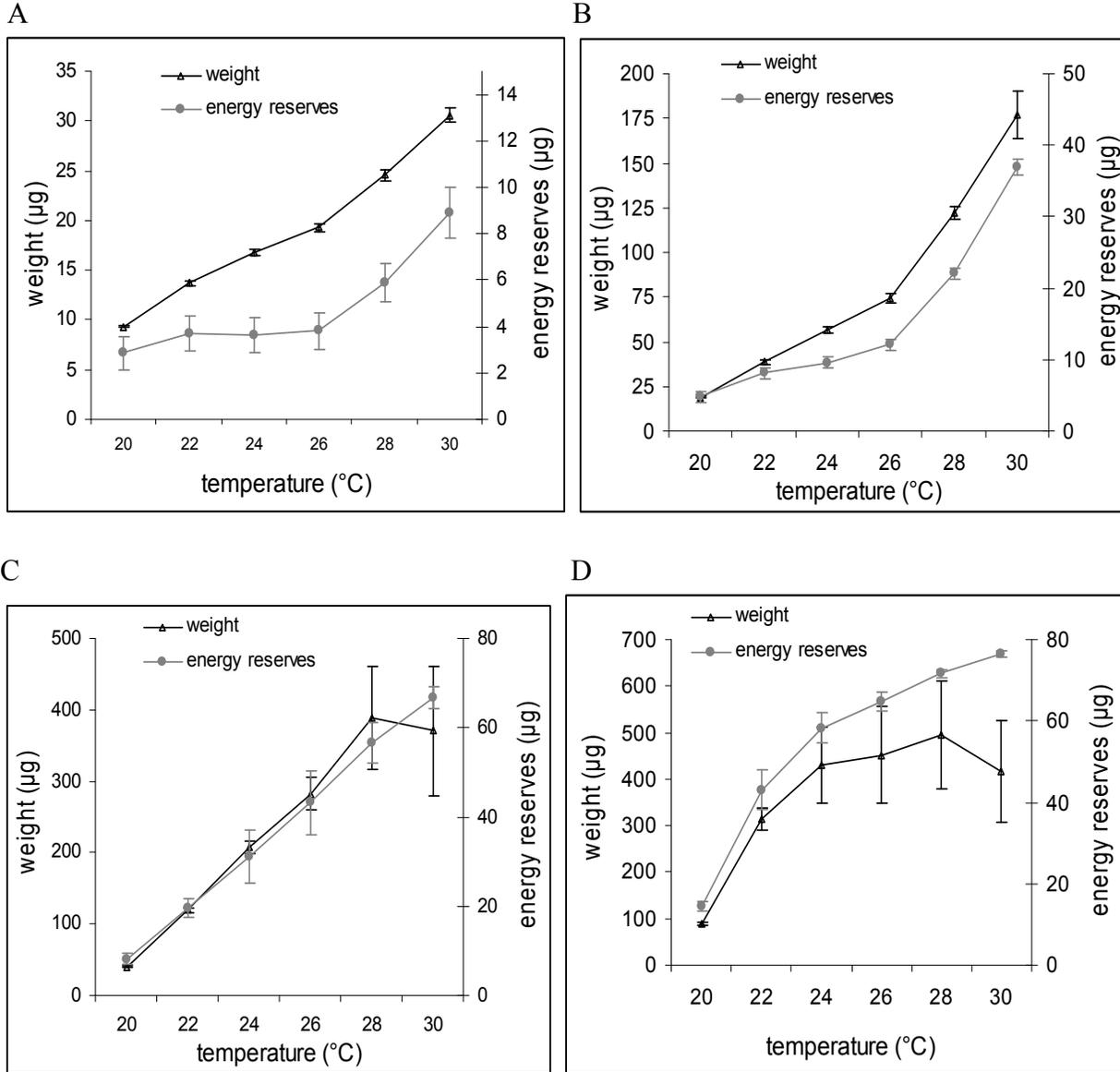


Figure 4-14. Simulated ML trajectories of mass and energy stores across temperature. A) 1 day-fed larvae. B) 2 day-fed larvae. C) 3 day-fed larvae. D) 4 day-fed larvae. E) 5-day fed larvae. Error bars represent standard error in a simulation of 4000 larvae.

E

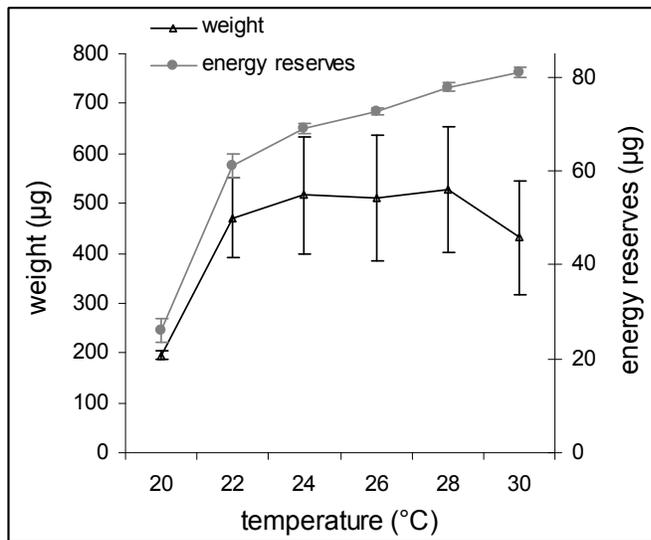


Figure 4-14. Continued

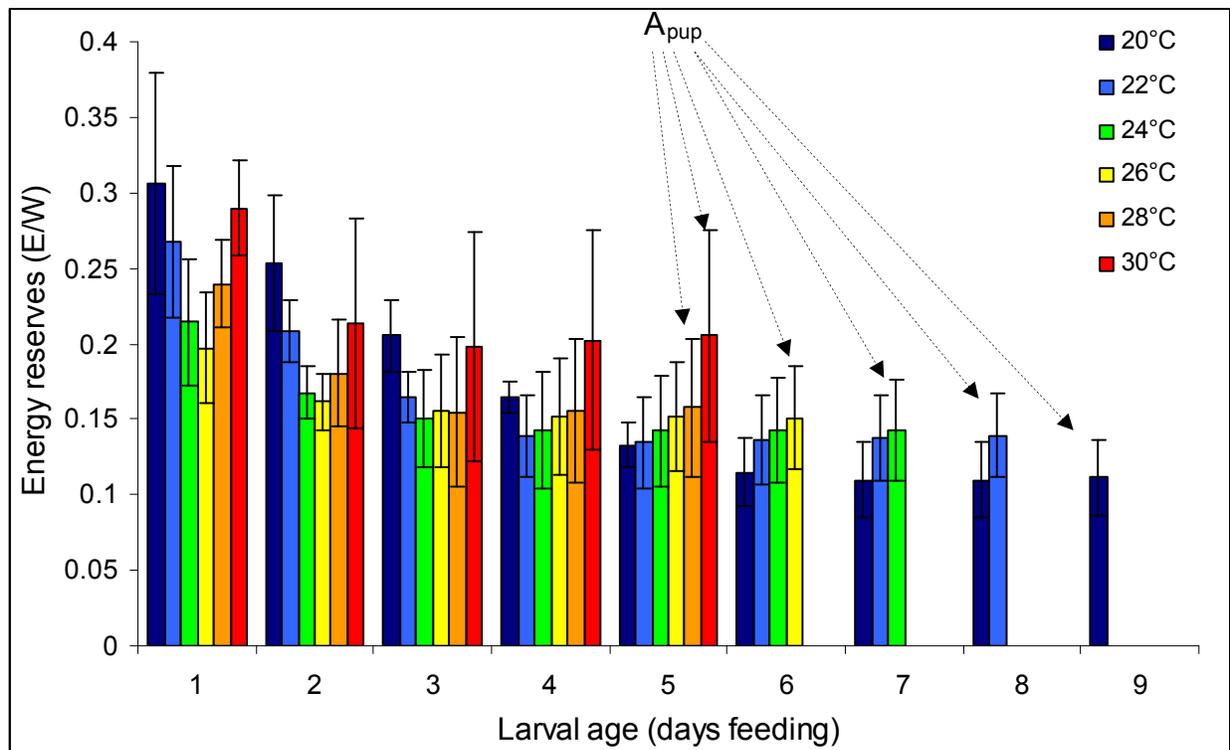


Figure 4-15. Simulated energy stores as a fraction of body weight across temperature and feeding age using ML parameter scenarios for each temperature. Error bars represent 95% CIs in a simulation of 4000 larvae.  $A_{pup}$  the laboratory observed median days to pupation (Experiment A) is the last bar for each temperature as highlighted by the arrows.

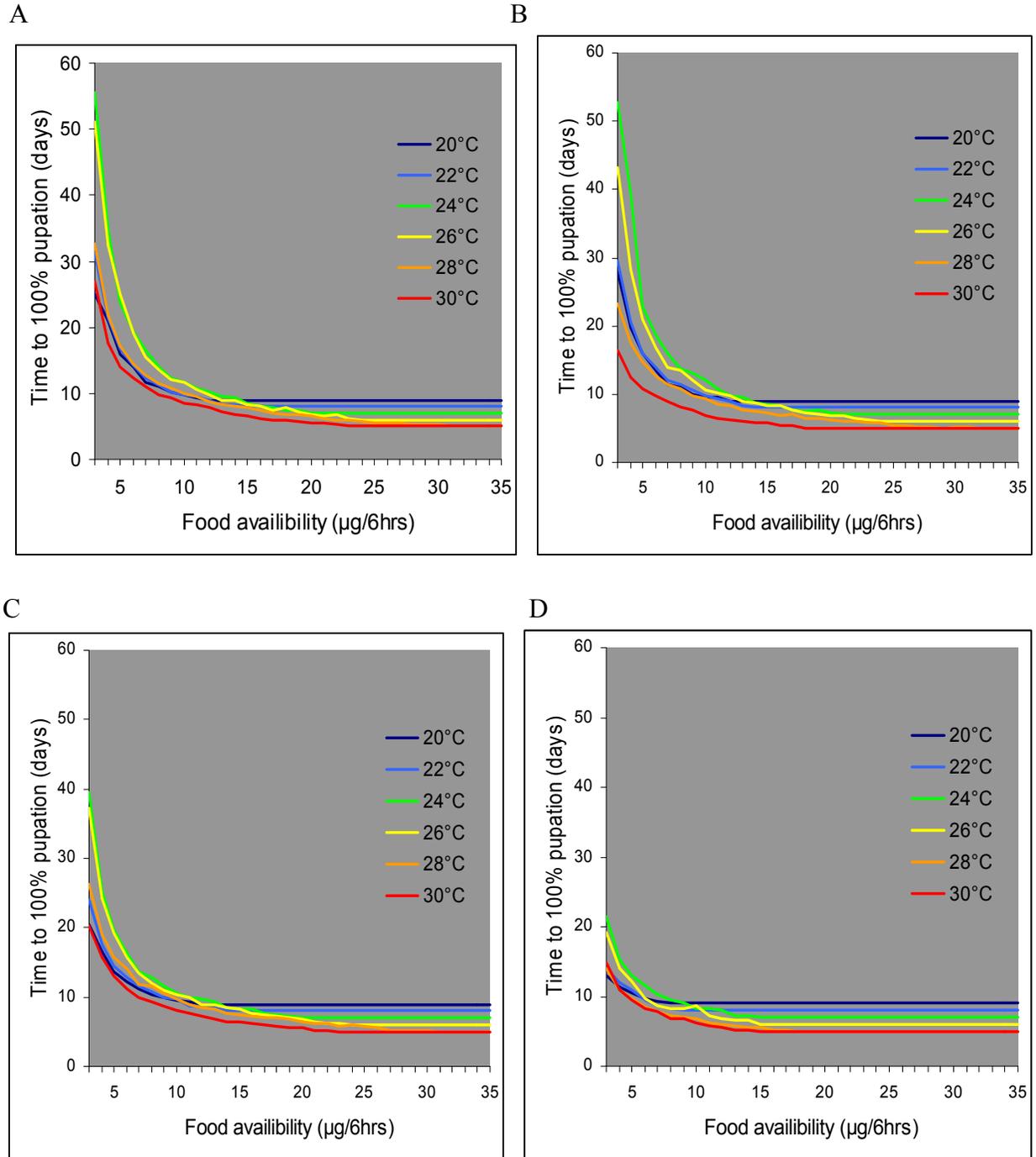


Figure 4-16. Food limitation and time to attain 100% pupation. In the first two panels larvae at all temperatures are food limited (A) immediately after hatching or (B) after feeding with excess food for 2 days. In C and D, larvae feed in excess for different times at each temperature until reaching (C)  $E_{th}$  or (D) the midpoint between  $E_{th}$  and  $E_{pup}$ . X-axis begins at  $3\mu\text{g}/6\text{h}$  in all panels, the lowest food level in which pupation is possible within 60 days in all four developmental scenarios.

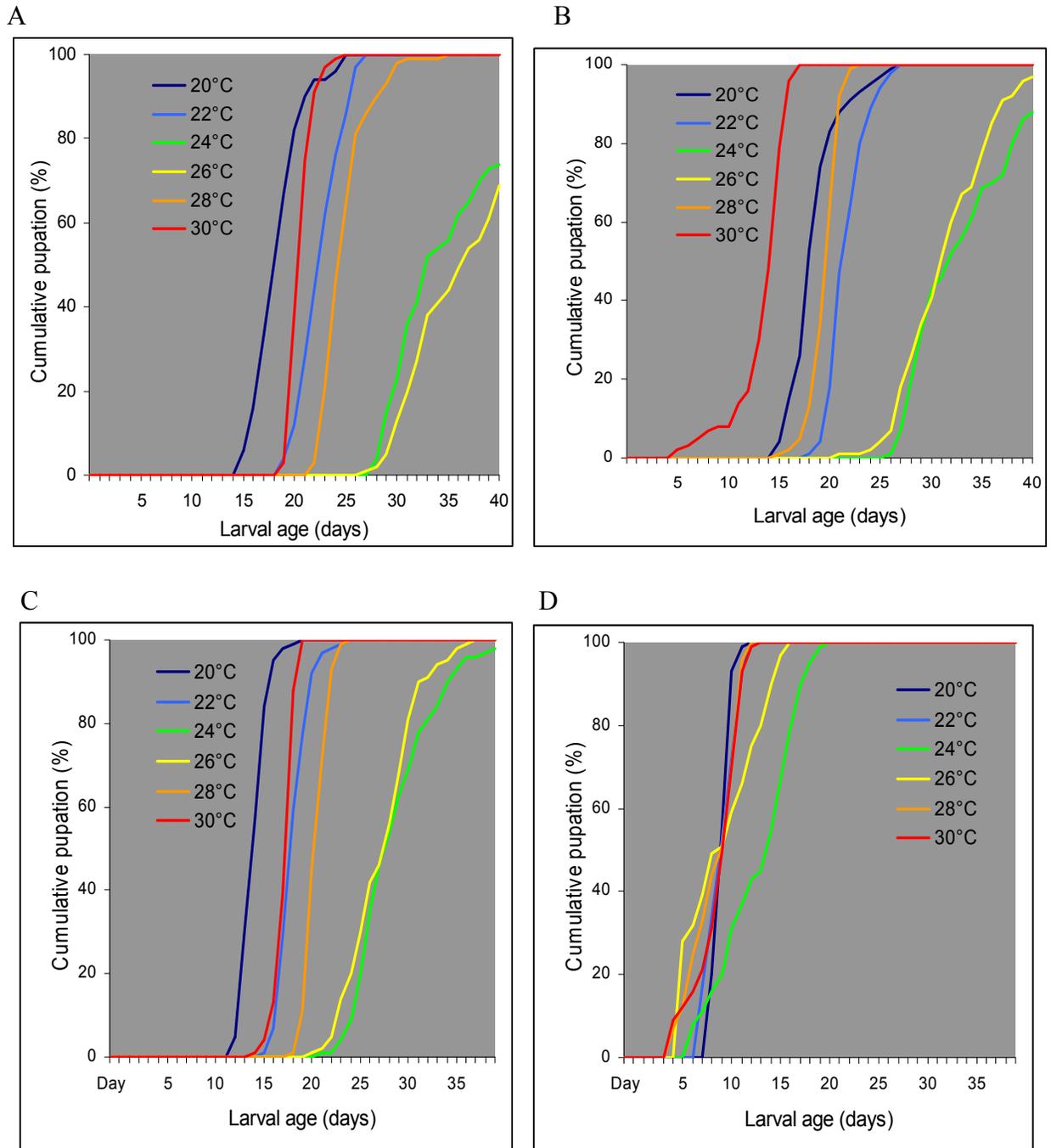


Figure 4-17. Effects of temperature on cumulative pupation under food limited conditions ( $3\mu\text{g}/6\text{h}$ ) In the first two panels larvae at all temperatures are food limited (A) immediately after hatching or (B) after feeding with excess food for 2 days. In C and D, larvae feed in excess for different times at each temperature until reaching (C)  $E_{th}$  or (D) the midpoint between  $E_{th}$  and  $E_{pup}$ .

## CHAPTER 5 CONCLUSIONS

In Chapters 2-4 we explored how temperature variation in a range observed in dengue endemic areas in Colombia interacts with a number of ecological processes that drive the production of domestic *A. aegypti* populations. The study system consists of socio-biological processes that interact with each other in response to abiotic conditions. Abiotic components include altitude, temperature, stored water, containers and larval food (ignoring that microorganisms consumed by larvae are living). The two “biotic” processes are (1) human filling, emptying, usage and maintenance of stored water in domestic vessels and (2) food assimilation and energy storage in *A. aegypti* larvae that allows growth and metamorphosis into pupae.

We have seen that the effects human-mediated and resource-mediated processes that determine the size, development rate and pupation success of *A. aegypti* can be modified across experimental temperature gradients or cities that vary in temperature. Moreover, we have developed a mechanistic framework that allows investigation of how *A. aegypti* responds to changing temperature and nutrient conditions that is amenable to future scaling-up to a community of vessels.

Figure 5-1 shows a schematic of the eco-social system under study. It depicts how the proximal environmental cues experienced by larvae are ultimately shaped by the interaction between climate and human-mediated habitat dynamics. Habitat resources include the abundance, location, environmental exposure and permanency of water-holding vessels, which, in turn, determine environmental determinants of larval development, such as water temperature, food availability and habitat stability. Moreover, these habitat features are themselves a product of human behavioral responses to variable environments. Key features of the human environment that engender water storage behaviors include the cost of water, the stability of

water service, variation in the structure of containers and local cultural norms, all of which may interact with individual perceptions, age, sex, income, etc.. A key feature of this system is that climate variation may have simultaneous direct and indirect impacts on mosquito development – either through its direct effects on water temperature or the abundance of water-holding vessels, or through its effects on human water storage behaviors. Below we discuss how *A. aegypti* larvae process specific cues in the household environment and ultimately how the interactions between larval and human ecology determines the development rate and size of emerging *A. aegypti*. We emphasize that human-ecological interactions are key features of the regulation of *A. aegypti* production among domestic vessels in dengue endemic neighborhoods of Colombia.

### **5.1 Interactions between the Container Environment and *A. aegypti* Growth and Development**

If, for simplicity, we overlook the potential for *A. aegypti* larvae to modify their environment through movement within containers, larvae are subject to a set of “externally” determined conditions determined by human activity that is ultimately responsible for the existence, location, permanency of all urban vessels. These conditions dictate a maximum time that a habitat will persist for larva to develop, as well as the temperature, nutrient and larval density conditions which determine biologically how much time a developing larva needs to pupate and emerge to adults.

Temperature variation is particularly acute among our Colombian field sites, as human-associated determinants of microclimate, including container material, water volume and sun exposure act over a range of altitudes. By fitting a novel developmental model to experimental data, we explored how larvae are likely to incorporate water temperature into the processes of growth and development. We showed that larvae exposed to different environmental conditions may exhibit changes in the initial growth rate, final growth rate and/or the duration of growth.

While a positive linear impact of increasing temperature on a developmental parameter is likely due to faster biochemical reactions, tradeoffs between development rate, size, fecundity and/or survival may cause mosquitoes to respond by modifying a facet of development that does not directly act through the enzyme-kinetics mechanism (Angilleta and Dunham, 2004; Kozlowski et al, 2004). Our results suggest that while heightened metabolism and food assimilation rate are “inevitable” at higher temperatures (within the 20-30°C optima), mosquitoes can compensate for negative impacts by modifying the duration and timing of growth, through changes in how they distribute acquired resources. In particular, by supposing that increased energy stores cause larvae to reduce food assimilation in the latter stages of development, we show that mosquitoes may reduce their final feeding rate; moreover, this reduction can generate the negative association between temperature and adult size commonly observed in mosquitoes and most ectotherm species.

Throughout the preceding chapters we emphasize that large variation in the resources available to *A. aegypti* across domestic vessels in a particular human community is well documented. We saw that the tradeoffs in temperature-induced increases in growth and metabolic rate may be more acute when resources are absent, as temperature increases maintenance costs of larvae; this compels *A. aegypti* larvae to increase the accumulation of energy reserves in order to ensure pupation. We found that if increased energy allocation occurs at the beginning of development it may significantly reduce the negative effects of food limitation on development rate and pupation success. However, if temperature-associated increases in energy storage occur at the end of development, they may result in a reduction in development rate in situations of food limitation. Indeed, both model predictions based on ML fits to experimental data on starvation resistance, and simultaneous manipulation of food and

temperature conditions in developing larvae, indicated that the developmental response of larvae to variation in resource conditions may be modified by temperature. In particular at 28°C increased larval resources may favor cell size more than cell number, whereas at 22°C heightened resources produce a larger increase in cell number.

Assuming that the resources available to larvae in water storage vessels are similar across the Colombian landscape, a 2°C seasonal or ENSO-associated temperature shift may have starkly different effects on the *A. aegypti* production among cities with different altitudes in the 0-1500 m range. Moreover, given evidence that spatial variation in vector production is related to resource availability (Barrera et al, 2006), temperature changes may also have differential impacts on the spatial distribution of emerging mosquitoes across altitudes. This is due to the fact that the characteristic aggregation of *A. aegypti* pupae in a few vessels in a community (Getis et al, 2003) may be linked to resource limitation in a majority of vessels (Barrera et al, 2006). Thus, if a 2°C temperature shift were to alleviate resource limitation at certain mean temperatures (and altitudes) but not others, there would be a differential impact on the spatial distribution of pupae and potentially on human exposure to dengue vectors.

A third aspect of *A. aegypti*'s response to container conditions is the interaction of larvae with each other. Indeed, in order to scale up our model to predict *A. aegypti* production dynamics in a community of vessels, it will be first necessary to model how conspecific larvae compete for resources. Notwithstanding the potential for variation in search efficiency among larvae, our model predicts that larvae will exert the largest competitive pressure, i.e. consume the most food, in the early and middle portions of the fourth instar when larvae possess only 50 to 75% of their final weight under optimal food conditions. Food assimilation rate decreases in the latter half of the fourth instar with increasing energy reserves. By contrast, other models of competition

(Gilpin and McClelland, 1979; Focks et al., 1993) assume that competition is temperature independent and that larvae monotonically increase food consumption with weight. Once food searching is incorporated into the model, our framework is likely to generate novel insights into the dynamics of intra-specific resource competition in *A. aegypti*.

## **5.2 Human Ecological Interactions and the Dynamics of *A. aegypti* Production**

As container water is purposefully stored for potential human use, larvae that inhabit domestic vessels are subject to a constant risk of being washed away or desiccated through emptying events. Simultaneously, however, human use and replenishment of water allows eggs to be deposited and exposed to a regular hatching stimulus throughout the height of the vessel. This contrasts from treehole mosquito habitats or large abandoned vessels that depend only on rainfall for variation in water level; these may be susceptible to seasonal or climate change induced droughts in which large egg banks may accumulate at certain levels with little egg hatching. By contrast, in vessels in which water is regularly extracted and replenished due to usage for common household activities such as cleaning and cloth washing, larvae are likely to hatch more frequently in smaller and irregular cohorts; this could potentially alleviate resource competition and increase the likelihood of continuous presence of a larva. Accordingly, in residential areas with high levels of water storage and usage of stored water, we may expect an increased abundance and frequency of larval infestation. For example in our study areas in Bucaramanga and Armenia where greater than 90% of households store water, we readily find that 20 to 30% of premises are infested with *A. aegypti* larvae at a given time.

In Chapter 2 we saw that the key process that mediates the impact of water use on *A. aegypti* production is water emptying. Emptying flushes both larvae and nutrients in containers; because larvae hatch faster than nutrients accumulate with water replenishment, emptying may simultaneously increase *both* larval mortality and density-dependent resource competition. Thus

frequently emptied vessels are more likely to demonstrate an interaction between (1) the impacts of temperature-food interaction on development rate discussed above, and (2) the upper limit to *A. aegypti* development time set by average emptying frequency. This supposition ignores the non-zero probability that a larva may survive an emptying event. Nonetheless, we found that on average, more frequent emptying was required to reduce pupal production in the warmer cities. Moreover water storage vessels that experienced emptying in Bucaramanga (24-25°C) had a mean production rate approximately 50% of those in Armenia (21-22°C) and Barranquilla (27-30°C), consistent with model predictions for food limited vessels. Moreover, our preliminary modeling studies (not presented here), parameterized using the associations between emptying frequency and pupal production rate described Chapter 2, suggest that stochastic water emptying that is independent among houses can explain the characteristic aggregation in *A. aegypti* production.

We found that human water storage behavior is largely determined through individual interactions with the physical and sociological environment. For example residents who store water not for regular usage but rather in case of interruptions in piped water supply are less likely to empty vessels. By contrast, emptying is more frequent in containers whose water is regularly extracted – as residents are more likely to empty when the water level reaches a minimum. All of our study neighborhoods have a stable water supply with occasional interruptions that are seldom prolonged for more than a few hours. However, we observed a universally lower frequency of emptying and higher *A. aegypti* production rate in the dry season, when interruptions in piped service are presumably or perceived to be more frequent. Thus, human adaptation to climate variation, rather than the direct impact of climate itself, may drive the association between climate and vector production (Beebe et al., 2009). Elderly people tended to perceive a greater

risk of going without water and were more likely to store water “just in case.” This is likely an outcome of customs developed over many years or multiple generations of living without a continuous water supply that are not easily modified when efficient water delivery systems are built. Interestingly, climate change, which is predicted to reduce rainfall and increase water insecurity in all three study cities, may proceed at a similar time-scale as cultural adaptation. This would suggest that the customs of the elderly may once again take hold in younger generations.

Not only cultural practices, but the structure of the vessel itself may influence the motivations and patterns of water storage behavior. In houses with permanent washbasins, residents don’t have to actively decide to store water and obtain a vessel, but rather, they use the vessel that came “built-in” as part of the house. Culturally, in Colombia hand-washing of undergarments is commonplace even in household with washing machines. In order to save water and for convenience residents almost universally opt to use water stored in the attached washbasin to scrub clothes instead of taking tap water. Thus, the motivation for water storage is primarily for the convenience of using stored water to wash clothes and secondarily for other reasons, such as interruptions in water service. In areas without built in wash-basins, by contrast, households must purposefully obtain a vessel and thus water storage is less frequent; moreover stored water often goes without usage. Unlidded, unused water often contributes to the accumulation of *A. aegypti* egg banks with less frequent hatching stimuli. In the face of increasing drought conditions, unstable water supply and decreased incentive to reduce water storage throughout Colombia, our data indicate that lid placement can be effective method of reducing *A. aegypti* production. We consistently observed less debris, although not necessarily lower larval infestation rates in lidded vessels, suggesting that the major action of lids is to

reduce the input of larval nutrients. However the structure of permanent washbasin makes lid placement on these containers extremely cumbersome and uncommon.

In short, sustainable management of *A. aegypti* production in changing urban environments requires understanding variation in motivation for household water storage behavior. Achieving such knowledge will require the use of social science methods capable of addressing the complex interactions between individual perceptions and the socio-economic, cultural and physical features of the human environment. In the preceding work we have moved forward towards developing a framework to study the *joint* interaction of the three separate ecological relationships: human behavior-*A. aegypti* production, climate-*A. aegypti* production and climate-human behavior. Understanding how this joint interaction plays out in human communities is the key to being able to apply our findings to predict the dynamics of *A. aegypti* production in changing environments.

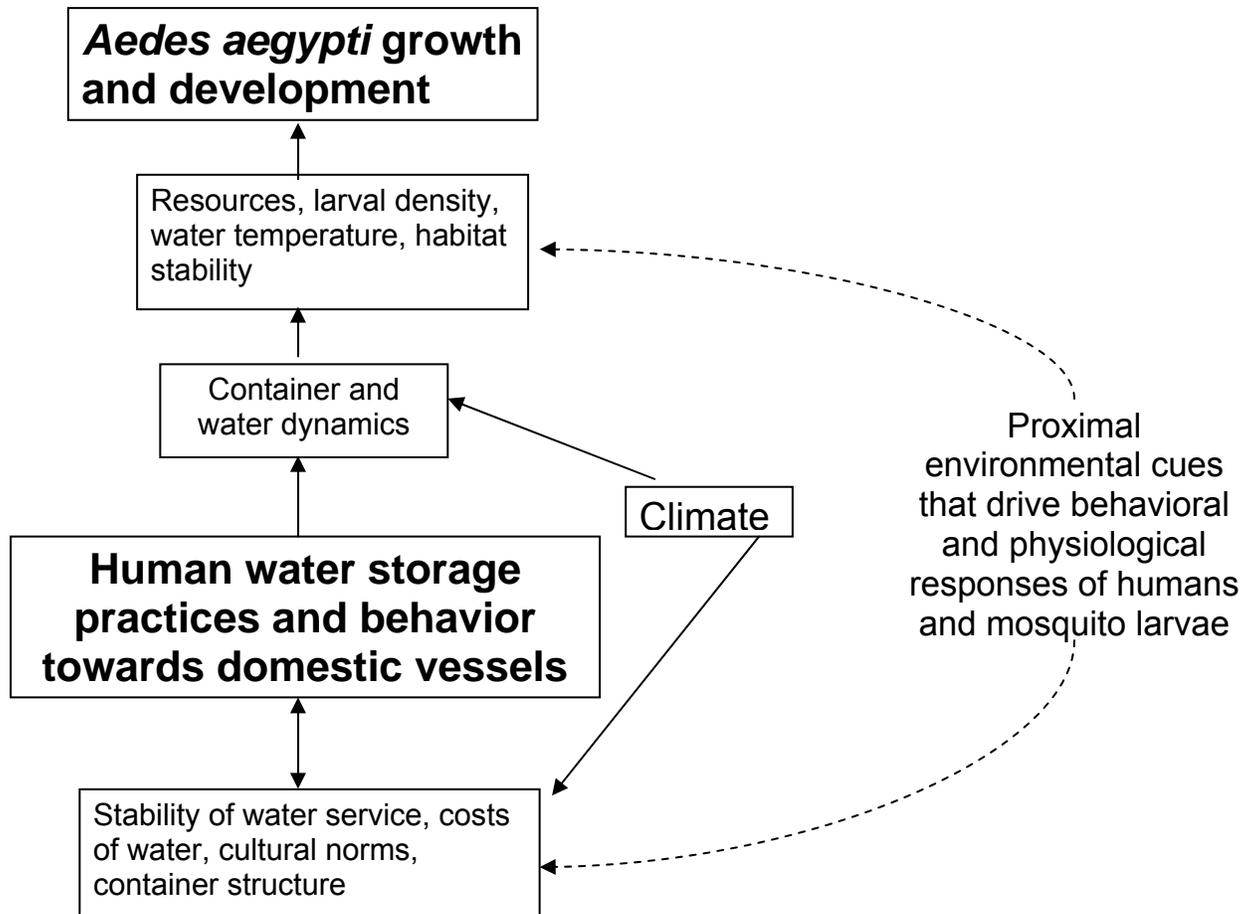


Figure 5-1. Schematic of eco-social system of domestic *Aedes aegypti* (L.) production.

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Harish Padmanabha leads a World Bank funded project on climate change and dengue ecology in Colombia, based in the Instituto Nacional de Salud of the Colombian Ministry of Health. For 8 years he has worked in the ecology and control of emerging diseases such as dengue, hantavirus, leptospira and spotted fever rickettsia in Panama, Cuba and Colombia, through appointments in institutions such as the Panamerican Health Organization (PAHO), National Institute of Health of Colombia, local health departments in Colombia, the Gorgas Institute and the Pedro Kouri Tropical Medicine Institute. He has an MSc in Population Sciences and International Health from Harvard University. He lives with his wife and two children in Barranquilla, Colombia.