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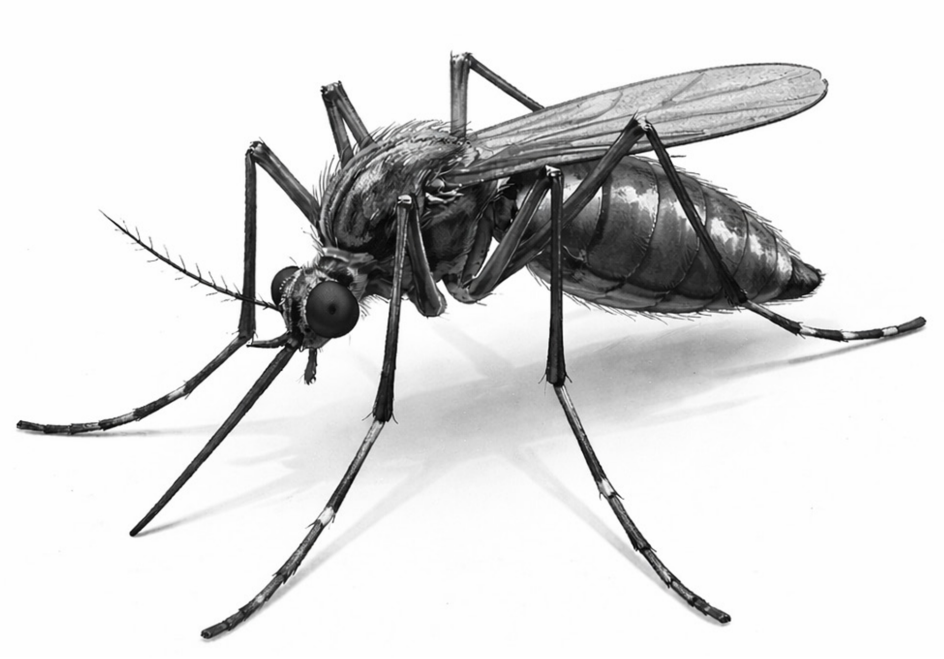
The effect of biotic and abiotic stress on mosquito fitness and behaviour

-a tale of four species

JULIAH WANJIRU JACOB



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The effect of biotic and abiotic stress on mosquito fitness and behaviour - a tale of four species

Abstract

The effect of temperature on fitness and behaviour of medically important mosquitoes has predominantly been evaluated under constant temperatures, yet in their natural habitats, mosquitoes experience fluctuating temperatures, and this may be more extreme under future changing climates. Food resource abundance in aquatic habitats fluctuates with temperature. How these two environmental factors interact to affect the life history of con-specific and hetero-specific mosquitoes during aquatic development, and fitness benefits carried over to adults is underexplored. Therefore, larvae of *Aedes aegypti*, *Anopheles stephensi*, *Anopheles coluzzii* and *Anopheles arabiensis* were reared under varying diurnal fluctuating temperatures and maintained on different larval resource levels (paper I and II). In addition, the effects of temperature on dynamic feeding of female *An. coluzzii* was evaluated (paper III). Shortened time to adult emergence, diminished adult size and longevity were observed at high temperature, in a resource- and genera-dependent manner (paper I and II). Moreover, teneral metabolic reserves carried-over to adults, as well as feeding propensity of teneral females, varied across temperature and resource regimes, in a genera-dependent manner (paper I). *Aedes aegypti* was a dominant competitor due to accelerated adult emergence, bigger sized females and longer adult survival compared to *An. stephensi* (paper II). Moreover, at low temperature, *An. coluzzii* was a dominant competitor with longer survival compared to *An. arabiensis* (paper II). Dynamic feeding of *An. coluzzii* on carbohydrate-, nitrogen- or proteinaceous-rich meal varied with female maturation, in a temperature-dependent manner (paper III). Findings from this study underscore the effects of biotic and abiotic stress on mosquito fitness traits and behaviour that are key to population growth rate, community structure and vectorial capacity.

Keywords: Carry-over, biotic and abiotic stress, dynamic feeding, fitness traits, resource level, mosquitoes, temperature, climate change.

Effekter av biotisk och abiotisk stress på myggors fitness och beteende - en berättelse om fyra arter

Abstract

Effekten av temperatur på kondition och beteende hos medicinskt viktiga myggor har huvudsakligen utvärderats under konstanta temperaturförhållanden, trots att myggor i sina naturliga habitat utsätts för fluktuerande temperaturer, och detta kan förväntas bli mer extremt under framtida förändrade klimat. Dessutom varierar födoresurserförekomst i akvatiska miljöer med temperaturen. Hur dessa två miljöfaktorer samverkar för att påverka livshistorien hos kon-pecifika och heterospecifika myggor under deras akvatiska utveckling, samt vilka konditionsfördelar som överförs till vuxenstadiet är otillräckligt utforskat. Därför odlades larver av *Aedes aegypti*, *Anopheles stephensi*, *Anopheles coluzzii* och *Anopheles arabiensis* under varierande dygnsfluktuerande temperaturer och med olika nivåer av larvresurser (artikel I och II). Dessutom undersöktes temperaturens effekt på dynamiskt födointaghos honar av *An. coluzzii* (artikel III). Förkortad tid till kläckning av vuxna, minskad kroppsstorlek och kortare livslängd hos vuxna observerades vid hög temperatur, på ett resurs- och släktesberoende sätt (artikel I och II). Dessutom varierade tenerala metaboliska reserver som fördes över till vuxna, liksom foderbenägenheten hos tenerala honor, mellan temperatur- och resursregimer, på ett släktesberoende sätt (artikel I). *Aedes aegypti* var en dominerande konkurrent på grund av snabbare utveckling till vuxenstadiet, större honor och längre livslängd jämfört med *An. stephensi* (artikel II). Vid låg temperatur, var däremot *An. coluzzii* en dominerande konkurrent med längre livslängd jämfört med *An. arabiensis* (artikel II). Det dynamiska födointaget hos *An. coluzzii* av kolhydrat-, kväve- eller proteinrika måltider varierade med honornas mognadsgrad på ett temperaturberoende sätt (artikel III). Resultaten från denna studie understryker effekterna av biotisk och abiotisk stress på myggors konditionsegenskaper och beteende, vilka är avgörande för populationstillväxt, samhällsstruktur och vektorkapacitet.

Nyckelord: Överföring, biotisk och abiotisk stress, dynamisk födointagning, konditionsegenskaper, resursnivå, myggor, temperatur, klimatförändringar

Dedication

To my beloved daughters: Precious and Gloria
Thank you for making this journey worthwhile, and for your silent prayers

"Carpe Diem"….Horace

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This thesis is based on the work contained in the following papers, referred to by Roman numerals in the text:

I. Juliah Wanjiru Jacob, Laura Grenville-Briggs and Merid Negash Getahun (2026). Diurnal fluctuating temperature and larval resource level interact to influence the life history and behaviour of disease-transmitting mosquitoes. Parasites and Vectors, 19 (150). https://doi.org/10.1186/s13071-026-07313-4.

II. Juliah Wanjiru Jacob, Merid Negash Getahun, Laura Grenville-Briggs, Robert Glinwood and Velemir Ninkovic (2026). Competition outcomes of sympatric mosquito species depend on the interaction between diurnal temperature fluctuations and larval resource level. Parasites and Vectors, (submitted manuscript).

III. Juliah Wanjiru Jacob, Velemir Ninkovic, Robert Glinwood, Laura Grenville-Briggs and Merid Negash Getahun. Age, temperature and their interaction govern the dynamic feeding of *Anopheles coluzzii* (manuscript).

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The contribution of Juliah Wanjiru Jacob to the papers included in this thesis was as follows:

I. Participated in designing the study, data collection and analysis, writing and revision of the manuscript.

II. Participated in designing the study, data collection and analysis, writing and revision of the manuscript.

III. Participated in designing the study, data collection and analysis, writing and revision of the manuscript.

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1. Introduction

Mosquitoes remain the most nefarious animals to mankind not only because of their nuisance biting (Halasa et al., 2014), but also due to pathogens transmitted (Hurd et al., 1995; World Health Organization, 2023) during ingestion of proteinacious blood meals which they use to foster propagation (De Swart et al., 2023; Valzania et al., 2019), and for metabolic energy (Briegel & Hörler, 1993; Takken & Verhulst, 2013). Despite concerted efforts targeting vector control, such as mass coverage with long-lasting insecticide treated nets, indoor residual spraying, larval source management, gene driven technology, attractive toxic sugar baits, endectocides, as well as therapeutics, morbidity and mortality associated with infectious mosquito bites continue to afflict millions of people globally (World Health Organization, 2023). The Achilles heel to these control arsenals, and elimination of mosquito borne diseases, is attributed to vector insecticide resistance, behavioural adaptation, pathogen resistance to drugs and climate change (IPCC, 2023; World Health Organization, 2023).

Climate change is the alteration of long-term weather patterns resulting in extreme weather events such as heat waves, drought, hurricanes and high precipitation (De Souza et al., 2024). Anthropogenic activities, *e.g.*, industrialization and urbanization, among others, are key drivers of climate change (De Souza et al., 2024; IPCC, 2023). Climatic factors play a crucial role in vectorial capacity, as well as spatial and temporal distribution of disease vectors (Brown et al., 2023; Caminade et al.,2019; Couper et al., 2021; Mordecai et al., 2019). For instance, humidity affects adult longevity and reproduction due to desiccation while precipitation determines success in breeding sites (Brown et al., 2023; Dao et al., 2014; Troyo et al., 2008). In addition, flooding due to high precipitation associated with El Nino Southern Oscillation has led to high incidences of Rift Valley fever due to an increase in vector density of floodwater mosquitoes in recent years, while malaria incidences reduced (Lindsay et al., 2000; Linthicum et al., 1999). Drought due to high temperatures has seen a rise in urban arboviral infections since *Aedes* mosquitoes utilise stored water in human dwellings for oviposition (Rose et al., 2020). Moreover, temperature unimodally affects fitness life history traits during the aquatic and terrestrial life stages of mosquitoes, as well as pathogens transmitted (Couper et al., 2021; Lahondère & Bonizzoni, 2022; Mordecai et al., 2017, 2019; Murdock et al., 2016; Villena et al., 2022). In recent times, global warming due to anthropogenic activities has exacerbated human suffering associated with mosquito borne diseases as a result of geographical niche expansion of disease vectors (Lahondère & Bonizzoni, 2022; Rose et al., 2020; Wilke et al., 2019; World Health Organization, 2023). Rising global temperatures increase the temperature safety margin for development of mosquitoes in cooler climates (Couper et al., 2021; Paaijmans et al., 2013). As such, regions previously devoid of mosquito borne diseases *e.g.*, dengue, malaria, zika, among others are now hotspots of these infections. Moreover, invasive species exert competition pressure on resident species, which affects vector population dynamics (Bevins, 2008; Juliano et al., 2005). Other reports from mechanistic modelling studies predict more than a billion naïve people are now at a risk of exposure to mosquito borne diseases (Ryan et al., 2019, 2021).

Successful thriving of mosquitoes is not solely dependent on abiotic factors but equally on biotic factors *e.g.*, food resource abundance in aquatic habitats, larval competition for food resources and predation (Carvajal-Lago et al., 2021; Juliano, 2009). Food resource abundance in aquatic habitats fluctuates with temperature and rainfall (Fillinger et al., 2009; Zettle et al., 2022). Moreover, metabolic energy demand of adult mosquitoes varies with temperature stress (Dillon et al., 2010), as such, foraging on sugar, blood (Costanzo & Occhino, 2023) and nitrogen-rich supplementary resources for metabolic energy and reproduction may vary across temperature gradients. While feeding is dynamic with age and physiological status of the insect (Alto et al., 2003; Foster, 1995; Gary & Foster, 2006; Hill et al., 2021; Takken & Knols, 1999), how temperature modulates dynamic feeding by mosquitoes remain to be elucidated. Overall, biotic and abiotic factors are crucial to fitness and physiology of mosquitoes, however, numerous studies evaluate each factor independently, yet in their lifetime mosquitoes experience all these factors together as a whole. Therefore, this study sought to evaluate how temperature interacts with biotic factors with an aim to provide a comprehensive understanding of their interplay in shaping life history fitness traits and behaviour of medically important mosquitoes, in view of global warming.

2. Background

2.1 The cycle of life

Mosquitoes exhibit two distinct yet intertwined life stages, in aquatic and terrestrial habitats. Eggs deposited by gravid females in ephemeral habitats e.g., containers, tires, rock pools, hoof prints and tree holes or perpetual habitats *e.g.*, agricultural fields, pools, dams and lakes hatch into larvae (Chandrasegaran et al., 2020; Clements, 1992). Larvae undergo four developmental instar stages, after which the fourth instar larvae metamorphosizes to pupae that transform into imagoes (Clements, 1992). Different mosquito species preferentially select their breeding sites, which may determine the level of biotic and abiotic stress experienced during aquatic development. For instance, the urban arboviral vector, *Aedes aegypti*, native to East Africa, is a container breeder which utilises flower vases, tires and discarded household containers (Rose et al., 2020; Leisnham et al., 2014). However, while the urban malaria vector, *Anopheles stephensi*, native to Asia, breeds in containers, this species utilises other large water bodies such as overhead tanks and cisterns (Taylor et al., 2024). The rural malaria vector, *Anopheles arabiensis* and the sibling rural and peri-urban species, *Anopheles coluzzii*, found in sub-Saharan Africa, predominantly breed in small open sunlit water pools and agricultural fields (Coetzee et al., 2000; Fillinger et al., 2009). Biotic, *e.g.* larval nutrition and competition, and abiotic, *e.g.* temperature and precipitation, factors in the aquatic habitat shape the transstadial fitness benefits *e.g.* size, survival and metabolic reserves carried-over to terrestrial adult stage of mosquitoes, and their ensuing behaviour *e.g.* blood feeding (Chandrasegaran et al., 2020).

2.2 Temperature

Mosquitoes are ectotherms whose body temperature fluctuates with environmental temperature (Paaijmans et al., 2013). Therefore, temperature plays an integral role in life history traits, ecology, vectorial competence, as well as the spatial, temporal and geographical range distribution of mosquitoes (Couper et al., 2021; Mordecai et al., 2019). For example, temperatures experienced in aquatic habitats determine egg hatchability, rates of development, larval stage duration and survival, and consequently, the ensuing adult size, longevity and fecundity (Barreaux et al., 2018; Chandrasegaran et al., 2020; Christiansen-Jucht et al., 2014, 2015; Couper et al., 2021; Couret et al., 2014; Ezeakacha & Yee, 2019; Mackay et al., 2023). In addition, temperatures experienced by adults in their terrestrial habitats modulate rates of metabolism, host seeking, blood feeding, biting activity, immunity and pathogen extrinsic incubation period (Barr et al., 2024, 2025; Costanzo & Occhino, 2023; Lahondère et al., 2023, 2025; Murdock et al., 2012; Shapiro et al., 2017; Villena et al., 2022; Winokur et al., 2020).

Owing to anthropogenic activities such as urbanization, change in land use, greenhouse gas emission and industrialization, a significant rise in global surface temperature by 1.4 °C compared to the pre-industrial period has been reported (IPCC, 2023). This has contributed to the altitudinal shift, as well as geographical range expansion of different medically important mosquito species. For instance, the malaria mosquito, *Anopheles arabiensis*, has gradually shifted to higher altitudes of Mt. Kilimanjaro (Kulkarni et al., 2016), while in other countries, highland malaria incidents are increasingly reported (Himeidan & Kweka, 2012; Siraj et al., 2014). Moreover, invasive species such as the Asian urban malaria mosquito, *Anopheles stephensi*, expanded its range to warming cities of the African continent (Kweka, 2022; Samake et al., 2025; Sinka et al., 2020; Taylor et al., 2024). Encroachment of new habitats and human dwellings by dengue and chikungunya vectors, *Aedes albopictus* and *Aedes aegypti* has also been reported (Radici et al., 2025; Rose et al., 2020; Ryan et al., 2019; Wilke et al., 2019), while modelling studies predict more than a billion people are at risk of *Aedes*-borne viruses infection (Ryan et al., 2019, 2021).

Despite extensive research on unimodal effects of temperature on fitness life history traits of disease vectors, most studies focus on effects of mean daily or monthly constant temperatures, yet due to complex life stages and behaviour, mosquitoes experience a plethora of temperature ranges in their natural habitats (Beck-Johnson et al., 2017; Paaijmans et al., 2013). Temperatures experienced by larvae in the aquatic habitats, for example, vary depending on the size of the water body (Paaijmans et al., 2008). Ephemeral sites such as containers, tree holes, rock pools or hoof prints have higher daily temperature fluctuations compared to perpetual sites such as dams or lakes (Chandrasegaran et al., 2020; Paaijmans et al., 2008). In addition, temperature ranges experienced while foraging, and in resting sites are dynamic (Paaijmans et al., 2013; Sauer et al., 2021). While recent reports incorporating effects of diurnal temperature fluctuations render support to effects of constant temperature on some life history traits (Evans et al., 2021; Huxley et al., 2021, 2022; Lyons et al., 2013), disparities in optimal temperatures for disease transmission have been reported. For example, future temperature ranges projected to support malaria transmission are lower than previously thought (Mordecai et al., 2013). Such differences are attributed to limits in critical temperature minima and maxima within geographical ranges of mosquito species (Beck-Johnson et al., 2017; Mordecai et al., 2013, 2019). Temperate species experience high seasonal temperature fluctuations and hence, have a broader temperature safety margin while tropical species experience low fluctuations, hence a narrow safety margin (Couper et al., 2024.; Deutsch et al., 2008; Oliveira et al., 2021). Therefore, using fluctuating temperatures will provide a better understanding of how mosquito life history fitness parameters, physiology, behaviour and vectorial capacity vary with temperature stress, and hence how global warming is defining disease transmission.

2.3 Food resources

Food resource availability and abundance is integral for somatic metabolic fuel required for survival, and to cope with temperature stress encountered during the aquatic and terrestrial life stages of mosquitoes (Huey & Kingsolver, 2019; Huxley et al., 2021, 2022; Padmanabha et al., 2011). Therefore, food resource abundance influences life history traits during larval stages and subsequent fitness benefits carried over to the adults (Evans et al., 2020; McCormick & Gagliano, 2008.). Larvae from rich food resource habitats have a ‘silver spoon advantage’ and hence, grow faster and develop into bigger adults with a longer life span, higher fecundity, metabolic reserves, as well as higher vectorial capacity (Briegel, 1990a, 1990b;

Carvajal-Lago et al., 2021; Couret et al., 2014; Evans et al., 2020; Huxley et al., 2021; Shapiro et al., 2016). While poor-food resource larval habitats are not beneficial to mosquitoes due to extended larval durations and increased predation (Huxley et al., 2021; Juliano, 2009), high disease transmission by adults from these habitats may be experienced. This is because of a high susceptibility to pathogens associated with diminished immunity and damaged mid-gut basal membrane, as well as increased biting rates to replenish metabolic reserves (Briegel & Hörler, 1993; Carvajal-Lago et al., 2021).

Food resources *e.g.* ectothermic microorganism, microbial break down products and plant detritus, *etc* (Merritt et al., 1992) are dynamic with temperature of aquatic habitats (Cross et al., 2015; Fillinger et al., 2009; Zettle et al., 2022). However, how temperature and larval food abundance interact to shape different mosquito fitness life history traits during aquatic development and ensuing carry-over effects on adult traits, physiology and behaviour, remains scantly evaluated. For example, limited food resource during larval development significantly reduced population fitness thermal optimum, as well as survival and size of *Ae. aegypti* (Huxley et al., 2021, 2022). Therefore, understanding complex synergistic effects of environmental factors on mosquitoes is fundamental to their vectorial capacity.

2.4 Competition

Owing to the dynamic temperature stress of aquatic habitats, survival of the fittest is dependent on the competitive prowess for food resources among larvae of con-specific or hetero-specific mosquito species (Ezeakacha & Yee, 2019; Juliano, 2009; Leisnham et al., 2014; Lounibos et al., 2002; Moore & Whitacre, 1972; Paaijmans et al., 2008). In laboratory studies for example, competition for limited food resources, accentuated negative effects of temperature stress on rates of development, juvenile mortality, and ensuing adult size and survival of *Ae. aegypti* (Huxley et al., 2022). In other studies, high larval density negatively affected the development rate, larval survival, ensuing adult size and survival of *Ae. aegypti* (Couret et al., 2014) while varying larval densities of *Culex quinquefasciatus* negatively affected the size of adult *An. gambiae s.s* (Kweka et al., 2012).

Asymmetric competition for food resources by invasive sympatric species, associated with fitness benefits such as accelerated rates of development, big sized adults, longer survival, population growth rate and vectorial capacity of dominant species has been reported (Alto et al., 2005; Bevins, 2008; Carvajal-Lago et al., 2021; Juliano, 1998, 2010; Moore & Whitacre, 1972). For instance, *Aedes* species are dominant competitors compared to *Culex* and *Anopheles* species. When reared together, under varying temperature regimes and larval densities, *Ae. aegypti* was a dominant competitor compared to *Culex quinquefasciatus* and *An. stephensi* (Evans et al., 2021; Alto et al., 2005; Carrieri et al., 2003; Marini et al., 2017; Santana-Martínez et al., 2017). Moreover, competition significantly enhanced viral infection and dissemination in *Ae. albopictus* when reared with *Ae. aegypti*, and that of *Ochlerotatus triseriatus* in presence of *Ae. albopictus* (Bevins, 2008). In other studies, *Ae. albopictus* was a dominant competitor compared to *Ae. aegypti*, and in field studies, displacement of the later by the former species has been reported (Juliano, 2010; Kraemer et al., 2019; Lounibos et al., 2002; Reinhold et al., 2018). Spatial- and condition-specific segregation, however, has been implicated in enabling the coexistence of invasive dominant species with resident species. For instance, both *Ae. albopictus* and *Ae. aegypti* thrive during early to late rainy season but the former is predominant in rural areas and the later in urban areas, while *Cx. quinquefasciatus* only thrives during the rainy season, and utilizes broad breeding habitats (Leisnham et al., 2014). Occurrence of sibling species may vary in a spatial and temporal scale depending on temperature or rainfall, in which high temperature fluctuations in ephemeral aquatic habitats is favourable to the arid species, *An. arabiensis*, compared to humid prone *An. gambiae s.s* and *Anopheles funestus* (Kirby & Lindsay, 2009; Mala et al., 2011; Paaijmans et al., 2009). Taken together, competition for food resources during larval development, as well as temperature stress have significant implications in ensuing fitness benefits carried-over to adult insects, vectorial capacity and population structure of mosquitoes (Dillon et al., 2010; Huey & Kingsolver, 2019).

Asymmetric competition for food resources by sympatric species during larval development has been attributed to differences in feeding habits, in which *Aedes* species are shredders that forage throughout the water film while other species are mostly filter feeders (Merritt et al., 1992). Moreover, *Aedes* species are not only resistant to starvation, but also efficient in converting food biomass to long term metabolic reserves compared to *Anopheles* species (Briegel, 1990a, 1990b; Van Handel, 1965). Under meagre food resource conditions, *Aedes* species was implicated to supress development of heterospecifics using growth retardant factors (Moore & Whitacre, 1972), while niche segregation of aquatic habitats counters the negative effects of competition on less competitive species (Leisnham et al., 2014). Among sibling species, disparities in metabolic energy requirement for basal sustenance is significant to asymmetric competition. When *An. arabiensis* was reared together with a high density of *Anopheles gambiae sensu stricto*, the former species was disadvantaged by its high metabolic energy requirement (Schneider et al., 2000). Overall, disparities in competitive dominance among sympatric species is significant to shaping the co-occurrence patterns of different species, mosquito community structure along invasion fronts (Juliano, 2010; Leisnham et al., 2014; Livdahl & Willey, 1991) and disease transmission dynamics (Juliano & Philip Lounibos, 2005). Moreover, the unequivocal role of temperature in determining food resource abundance in aquatic habitats (Cross et al 2015; Fillinger et al., 2009; Zettle et al., 2022), has significant ramifications in shaping competitive outcomes among sympatric species, yet such wholistic interactions remain scantly evaluated (Evans et al., 2021). This underscores the significance of evaluating effects of biotic and abiotic factors, simulated to represent natural conditions, on immature stages in shaping fitness life history traits and disease transmission dynamics by invasive sympatric species.

2.5 Fuel of life

During the aquatic phagophase, mosquito larvae forage on available food resources to amass metabolic reserves for basal maintenance, as well as transitional energy across different life stages (Briegel, 1990a, 1990b; Briegel et al., 2001; Timmermann & Briegel, 1999). Protein forms the integral structural component, while free circulating glucose in the hemolymph is the primary energy source for survival and flight (Arrese & Soulages, 2010). Excess glucose is converted to long term reserves, glycogen and lipids, which are stored in the fat body. During starvation lipids are catabolised to metabolic water and glycogen, which is in turn broken down to glucose (Arrese & Soulages, 2010).

Accumulation of metabolic reserves varies among mosquito species, and is dependent on food resource abundance and quality, foraging habits, as well as temperature stress (Briegel, 1990a, 1990b; Merritt et al., 1992; Van Handel, 1965). For instance, adults of larvae fed on a protein- and fat-rich diet had higher lipids compared to those offered plant leaf diet, while a high food resource availability during larval development resulted in adults with a high caloric content (Timmermann & Briegel, 1996). In other studies, high constant temperature reduced the larval foraging duration and hence, a linear correlation between caloric content and size of adult *Ae. albopictus* was observed (Briegel & Timmermann, 2001). Moreover, shredders such as *Aedes* species larvae are efficient foragers and hence accumulate more reserves compared to *Anopheles* species (Briegel, 1990a; Merritt et al., 1992; Van Handel, 1965).

Metabolic reserves carried-over to the adult stages are integral to vector fitness and pathogen transmission (Briegel, 1990a, 1990b; Carvajal-Lago et al., 2021). Adult longevity and reproduction are directly related to metabolic reserves, in which teneral malnourished adults have a lower survival, mating and fecundity (Briegel, 1990b; Briegel et al., 2001). While food resource abundance and temperature affect metabolic reserve accumulation during larval development and subsequent adult fitness (Briegel, 1990b; Briegel et al., 2001; Briegel & Timmermann, 2001; Timmermann & Briegel, 1999), how the interaction of the two environmental factors, under simulated natural conditions, affect teneral reserves in different mosquito species remain to be elucidated.

2.6 Resource seeking and feeding

Male and female mosquitoes indulge in plant-seeking to replenish metabolic fuel for survival and reproduction from carbohydrate- and amino acid-rich floral nectar, extra-floral nectar, sap, fruits and honeydew (Barredo & DeGennaro, 2020; Fikrig et al., 2020; Foster, 1995; Peach & Gries, 2020; Shannon et al., 2024; Upshur et al., 2023). Moreover, females host-seek for energy and oogenesis (Hurd et al., 1995; Takken & Verhulst, 2013; Valzania et al., 2019). Odorant cues mediate preferential feeding by mosquitoes on beneficial sugar- and blood-hosts (Hill & Ignell, 2021; Nyasembe & Torto, 2014; Takken & Knols, 1999). In the absence of the two primary resources, nitrogenous meals such as cow urine and insect haemolymph supplement the energy requirements for basal sustenance and reproduction of mosquitoes (Dawit et al., 2022; George et al., 2014; Martel et al., 2011). While mosquitoes forage on sugar sources throughout their life, feeding by females is dynamic (Foster, 1995; Hill & Ignell, 2021). For instance, low metabolic reserves in newly emerged females, offered limited nutrition during larval development, increases their attractiveness to sugar compared to blood resources (Foster & Takken, 2004; Hancock & Foster, 1997). Upon accumulation of energy reserves and maturation of probing mouthparts, refractoriness to sugar and attractiveness to blood resource is observed in older females (Hancock & Foster, 1997; Hill & Ignell, 2021; Takken & Verhulst, 2013). Moreover, mating regulates cycles of blood feeding by females (Vinauger & Chandrasegaran, 2024). However, in energy depleted gravid females seeking an oviposition site, attractiveness to sugar source odours is restored (Hill et al., 2021; Hill & Ignell, 2021), while infection of female *An. gambiae s.s.* with *Plasmodium falciparum* increases their plant probing and feeding (Nyasembe et al., 2014).

Despite extensive efforts to understand how internal status of mosquitoes modulate dynamic host seeking and feeding, as well as molecular mechanisms associated (Hill et al., 2021; Hill & Ignell, 2021; Omondi et al., 2019), effect of external factors on these behaviours remain scantly elucidated. For instance, seasonal availability of plant- and animal-hosts determines frequency of sugar and blood feeding by mosquitoes in the field (Müller & Schlein, 2005; Upshur et al., 2023). In other studies, high constant temperature and low humidity increased flight activity, antennal response to host odours, attraction to host odours, biting activity and blood feeding of mature *Ae. aegypti*, while high temperature reduced blood feeding by *Ae. albopictus* (Costanzo & Occhino, 2023; Holmes et al., 2025; Lahondère et al., 2023). Although findings from these studies signify relevance of abiotic factors on mosquito behaviours key to disease transmission, only a single age group was targeted. In addition, how abiotic factors influence supplementary feeding from other nutritional resources *e.g.* urine, remain to be elucidated. As such, evaluating how ecologically relevant temperatures, as well as their interaction with age influence dynamic feeding of medically important mosquitoes is significant to a comprehensive understanding of mosquito behaviour in the context of global warming.

3. Aims and objectives

This study aimed to establish the effects of biotic and abiotic stress on fitness and behaviour of four mosquito species namely, *Aedes aegypti*, *Anopheles stephensi*, *Anopheles arabiensis* and *Anopheles coluzzii.*

The first objective was to evaluate the interactive effects of diurnal fluctuating temperature and larval resource level on immature development, and subsequent carry-over fitness benefits from larvae to adults of *Ae. aegypti*, *An. stephensi*, *An. arabiensis* and *An. coluzzii* (paper I).

The second objective was to investigate the interactive effects of diurnal fluctuating temperature and larval resource level on competition of sympatric mosquito species (paper II).

The third objective was to assess the effect of age, temperature and their interactions on dynamic feeding of *An. coluzzii* (paper III).

4. Materials and methods

4.1 Mosquito colony maintenance

Rearing conditions for colonies used in these studies were 12 h: 12 h light: dark photoperiod, 25 ± 2 °C and 65 ± 5% relative humidity. Adults of *Ae. aegypti* (Rockefeller), *An. stephensi* (type-form, SDA-500), *An. coluzzii* (G3) and *An. arabiensis* (Dongola) were maintained on 10% sucrose *ad libitum*, and defibrinated sheep blood (Håtunalab AB, Bro, Sweden) was offered to mature females via membrane feeding (Hemotek Ltd, Blackburn, UK). Oviposition substrate for gravid females constituted ovicups (30 ml; Nolato Hertila AB, Åstorp, Sweden) half filled with distilled water, and lined with filter paper (90 mm; Whatman®, Thermo Fischer Scientific, Gothenburg, Sweden). Filter papers were immersed in 1 L of distilled water in plastic trays (7 cm × 18 cm × 20 cm) to allow egg hatching. Emerged larvae were fed Tetramin® fish food (Tetra GmbH, Melle, Germany), after which pupae were pippeted into ovicups (30 ml; Nolato Hertila AB), and placed in Bugdorm cages (30 cm × 30 cm × 30 cm; MegaView Science Co., Ltd, Taichung, Taiwan). Emerging adults were maintained as detailed above.

4.2 Effect of diurnal fluctuating temperature and larval resource level on immature development and fitness benefits carried over to adults

4.2.1 Experimental design

A 3 × 3 × 4 factorial design, including three resource regimes, three fluctuating diurnal temperature ranges and four mosquito species (Figure 1A), was used to evaluate interactive effects of temperature and larval resource level (abiotic stresses) on fitness life history traits and feeding of disease vectors (paper I). Three temperature regimes, 22 ± 5 °C, 27 ± 5 °C and 32 ± 5 °C, were adopted from the geographical range distribution of *Ae. aegypti*, *An. stephensi*, *An. coluzzii* and *An. arabiensis* (Coetzee et al., 2000; Kraemer et al., 2015; Sinka, 2013, 2020). Experimental chambers (IPP750ecoplus; Memmert GmbH, Büchenbach, Germany) were programmed to generate a gradual increase in mean temperature up to a maximum during mid-day and a minimum just before sunrise. A photoperiod of 12 h: 12 h light: dark was used, and a 30 min gradual transition, at 30% light intensity, between photoperiods was adopted to simulate sunrise and sunset. Relative humidity ranged from 90-100%, and data loggers (Gemini Data Loggers Ltd, Chichester, UK) were used to monitor temperature and humidity in the climatic chambers throughout the experimental period (details in paper I).

The two invasive urban dengue and malaria transmitting species, *Ae. aegypti* and *An. stephensi*, respectively, share breeding habitats (Balkew et al., 2020), and hence larvae were maintained on a high resource level of 1 mg larva^{-1} day^{-1}. Previously, this resource regime supported a high larval survival and pupation of *Ae. aegypti* (Huxley et al., 2021). The effect of resource limitation was evaluated by scaling down the high resource level by a factor of $10^{0.5}$ to obtain a 'mid'-resource level of 0.32 mg larva^{-1} day^{-1}, as well as by a factor of 10^{1} to obtain a low resource level of 0.1 mg larva^{-1} day^{-1}. For the two malaria transmitting sibling species, *An. coluzzii* and *An. arabiensis*, 0.75 mg larva^{-1} day^{-1} was adopted as the high resource level based on its routine use in laboratory maintenance of the two species (Gerberg et al., 1994). Mid-and low-resource level regimes were obtained by scaling down high resource level, as described above, to 'mid' (0.24 mg larva^{-1} day^{-1}) and low (0.075 mg larva^{-1} day^{-1}) resource levels (paper I).

Eggs from each of the four species were hatched in distilled water previously incubated for 24 h in experimental chambers. First-instar larvae were pippeted into 1 L distilled water in cohorts of 50 per tray (7 cm × 18 cm × 20 cm) and replicated six times for each species across each temperature and resource regime. Larval mortality in each treatment was monitored daily, and amount of food adjusted to maintain a steady resource amount (mg larva^{-1} day^{-1}) for the duration of the experiment. To avoid toxin accumulation from microbial growth, and to maintain uniform food quantity, larval trays were cleaned and water changed daily (paper I).

4.2.2 Life history traits

Pupae from each replicate per treatment were collected into ovicups and placed in Bugdorm cages (MegaView Science). Duration from egg hatching to adult emergence was used to estimate the time to adult emergence of individual mosquitoes. Emerging adults were grouped as per the date of emergence and fed distilled water *ad libitum*. Resilience of the four species to abiotic stressors, associated with metabolic reserves carried over from their immature stages, was evaluated by measuring the survival of starved adults across different temperature and resource regimes. The size of females in each treatment was estimated from wing length (mm) (Van Den Heuvel, 1963) (paper I).

4.2.3 Metabolic macronutrient reserves

Fitness benefits carried-over from larval to adult stages were measured in the form of metabolic reserves in teneral adults from different temperature and resource regimes (Figure 1B). Amounts of total soluble protein, glycogen, carbohydrates and lipids were measured from <6 h old mosquitoes. Macronutrients from individual insects were fractionated as previously described by (Foray et al., 2012). Whole insects were macerated in protein lysis buffer. To estimate total soluble proteins, a fraction of the sample was mixed with Coomassie blue dye (250 µl, Bio-Rad Laboratories AB, Solna, Sweden) and absorbance was obtained at 595 nm (Multiskan FC, Thermo Fischer Scientific, Sweden). Known volumes of bovine serum albumin (Bio-Rad Laboratories AB) were used to estimate protein content from each insect (Bradford, 1976) (details in paper I).

The remaining fraction of the sample was fractionated in 20% sodium sulphate (20 µl, Merck Life Science AB, Solna, Sweden),1:1 methanol: chloroform (1.5 ml, both 99%, Merck Life Science AB) and 500 µl distilled water, and centrifuged at 6700 RCF for 5 min. The total carbohydrates in methanol and distilled water fraction formed the supernatant while lipids in chloroform formed the bottom layer. Hot anthrone reaction was used to analyse total carbohydrates at 620 nm Multiskan FC, Thermo Fisher Scientific). Lipids were analysed by vanillin reaction at 520 nm (Multiskan FC, Thermo Fisher Scientific) (Van Handel, 1985a, 1985b). Glycogen in the pellet was measured using the hot anthrone reaction at 620 nm (Multiskan FC, Thermo Fisher Scientific) (Van Handel, 1985a). To estimate the amounts of total carbohydrates and glycogen, known volumes of D-glucose (99.5%, Merck Life Science AB) were prepared in anthrone reagent and used to generate a calibration curve. In addition, lipid content was quantified using known volumes of olive oil prepared in vanillin reagent (99%, Merck Life Science AB) (Van Handel, 1985a; 1985b). To normalize for size biases, in each temperature and resource regime, amount of each metabolic macronutrient in individual insects was scaled by the respective average wing size (Timmermann & Briegel, 1999) (Paper I).

4.2.4 Feeding propensity of teneral females

Effects of temperature and larval resource on feeding propensity was evaluated in newly emerged females offered either a carbohydrate- (60% v/v bee honey; ICA Solna, Sweden) or protein-rich meal (sheep blood) (Figure 1C). Feeding propensity was conducted during photophase (Zeitgeber time, ZT, 2-5) and scotophase (ZT 13-16) analogous to *Ae. aegypti* and Anophelines diel-activity period, respectively (Foster & Takken, 2004; Jones, 1981). Honey mixed with 1 mg ml^{-1} xylene cyanol (Merck Life Science AB) was dispensed from 0.2 ml PCR strip tube caps (VWR, Stockholm, Sweden), and mosquitoes were allowed 3 h to feed. Blood was offered for 1 h via membrane feeding (Hemotek Ltd). Proportion of females ingesting honey were scored via blue colouration of the abdomen. Quantity of honey and blood ingested by females that fed was measured by spectrophotometry as described by (Dawit et al., 2022) and (Briegel et al., 1979), respectively. The volumes of each meal were scaled by the mean size of females, from respective temperature and diet regimes (paper I).

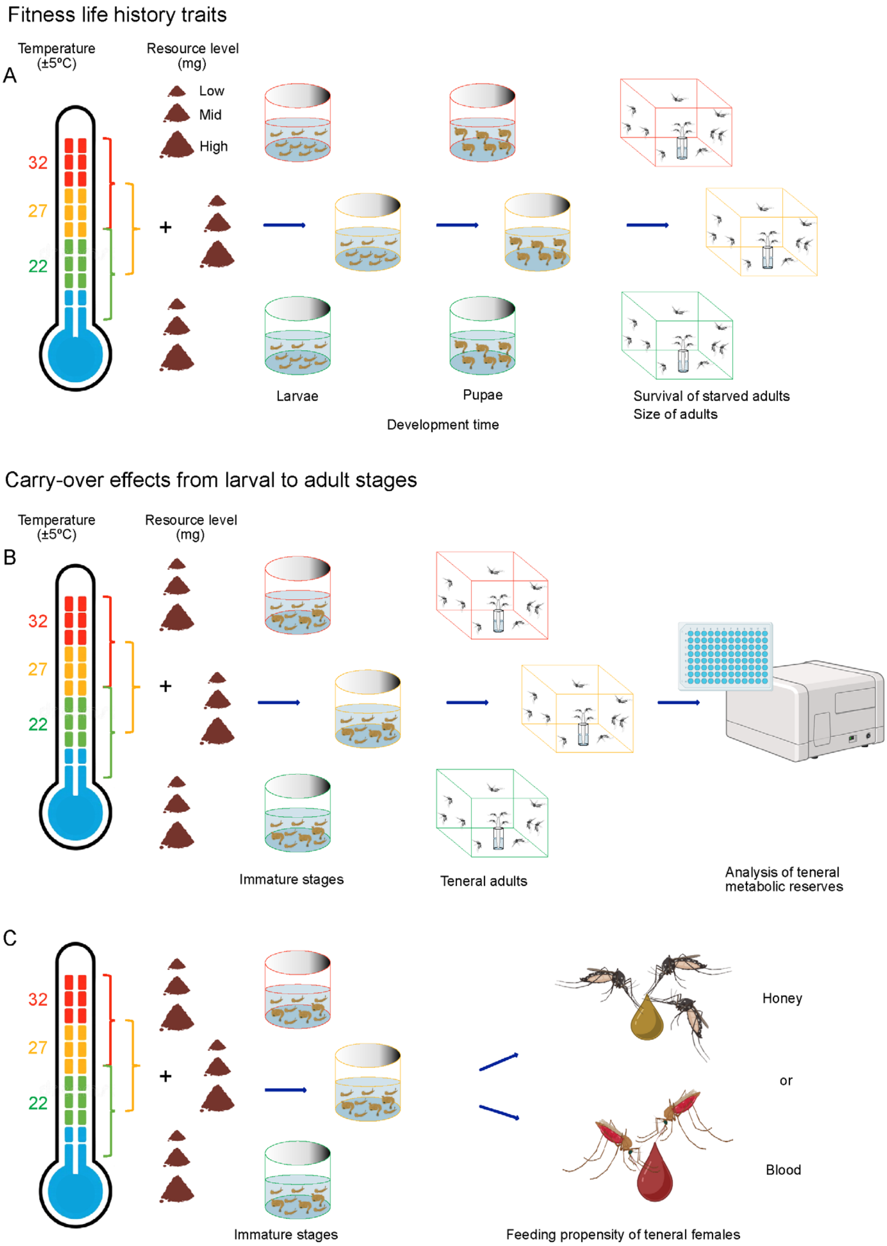


Figure 1. A schematic representation of experimental set up to study the effects of temperature and larval resource level on fitness traits of (A) immature and adult stages, (B) teneral metabolic reserves and (C) feeding propensity of teneral females (Jacob et al., 2026).

4.3 Effect of diurnal fluctuating temperature on larval competition for food resources by sympatric mosquito species

4.3.1 Experimental design

A 1:1 ratio of each species combination of *Ae. aegypti* and *An. stephensi*, as well as *An. coluzzii* and *An. arabiensis* larvae were maintained on either a high or low resource level and reared under three temperature regimes; 22 ± 5 °C, 27 ± 5 °C and 32 ± 5 °C. This resulted in a 2 × 2 × 3 factorial design, that was replicated six times for each temperature and diet regime (details in paper II). Conditions of experimental chambers were programmed as described above (see section 4.2.1).

To evaluate interspecies competition for food resource, cohorts of 25 1st instar larvae of each species were transferred into 1 L of distilled water in rearing plastic trays (7 cm × 18 cm × 20 cm). Larvae of the two urban (dengue and malaria) vectors, *Ae. aegypti* and *An. stephensi*, were maintained on either a high- (1 mg larva^{-1} day^{-1}) or low- (0.32 mg larva^{-1} day^{-1}) resource level (Huxley et al., 2021; paper I), while those of the two predominantly rural malaria vectors, *An. coluzzii* and *An. arabiensis*, were reared on 0.75 mg larva^{-1} day^{-1} or 0.24 mg larva^{-1} day^{-1} (Gerberg al., 1994; paper I). To maintain a constant resource level (mg larva^{-1} day^{-1}), daily removal of dead larvae was carried out and the amount of food adjusted per surviving individuals. To avoid microbial growth, as well as to maintain a constant amount of food, larval trays were cleaned and water changed daily (details in paper II).

4.3.2 Fitness life history traits

For each treatment and replicate, pupae were collected into ovicups (30 ml; Nolato Hertila AB), placed in Bugdorm cages and emerging adults were offered distilled water. Development time was estimated as duration from egg hatching to adult emergence of each mosquito. Polymerase chain reaction was used to distinguish between *An. coluzzii* and *An. arabiensis* (Scott et al., 1993). Adults were grouped as per the date of emergence and maintained on distilled water. Carry-over effects of temperature-dependent larval competition for food resources were measured from size and survival of starved adults. Size of adults from each treatment was estimated from wing length of males and females (Van Den Heuvel, 1963) (paper II).

4.4 Effect of age and temperature on dynamic feeding of *An. coluzzii*

4.4.1 Experimental design

Age-dependent feeding of *An. coluzzii* was first evaluated under constant ambient temperature. Experimental conditions were similar to those used in general colony maintenance (section 4.1). Larval density per tray was maintained at ca~150 and feeding regime on Tetramin fish food was ca~0.75 mg larva^{-1} day^{-1}, as described paper I and II. Pupae were collected into ovicups and placed in 15 cm × 15 cm × 15 cm Bugdorm cages. Emerging adults were age matched per the date of emergence into cohorts of 1, 3, 5, 6, 7, 8, 9 and 10 days old. Feeding propensity of teneral adults was evaluated on females maintained on distilled water. Older age groups were maintained on 10% sucrose *ad libitum*, and 24 hr prior to experimental age insects were starved on distilled water.

To evaluate how age-dependent feeding varies across different temperature regimes, feeding propensity of four age cohorts was evaluated. Mosquitoes were reared in experimental chambers under fluctuating temperature conditions as described in paper I and II. A cohort of 50 1st instar larvae were pippeted in 1 L distilled water and maintained on 0.75 mg larva^{-1} day^{-1} of Tetramin fish food, as previously described (paper I). Pupae were collected into ovicups, and emerging adults grouped per the date of emergence into experimental age cohorts of 1, 3, 7 and 10 days (paper III).

4.4.2 Feeding assays

For each age cohort, 20 females per cage were offered 10 % v/v bee honey (carbohydrate rich meal) for 3 h, blood (proteinaceous meal) for 1 hr and 1% 24 h aged cow urine (supplementary meal) for 12 hours. Feeding on different meals by each age cohort was replicated in ≥30 females. Moreover, feeding was conducted during scotophase (ZT 13-16) Dispensation of honey, urine and blood to mosquitoes, as well as estimation of meals ingested was done as described in paper I (section 4.2.4) (details in paper III).

5. Results summary and discussion

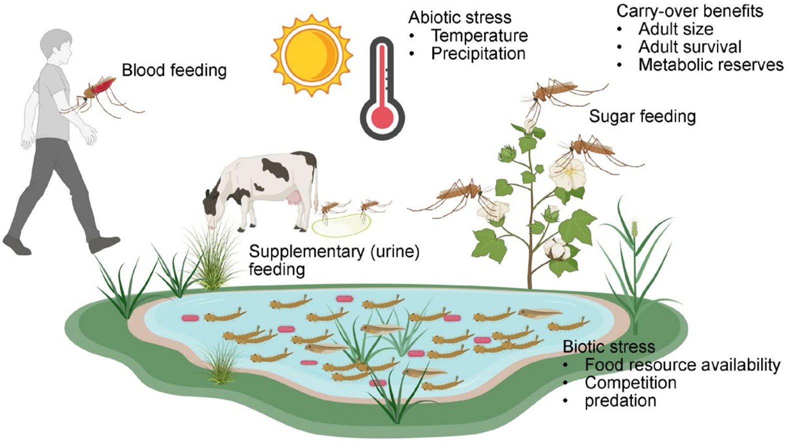


Figure 2. A schematic representation summarising biotic and abiotic stress experienced by mosquitoes. Stress during aquatic development determines ensuing fitness traits carried-over to terrestrial adult stages and behaviour (image created in BioRender.com).

5.1 Interactive effect of temperature and larval resource level on immature development

As poikilotherms, mosquitoes cannot rely on their metabolism for thermoregulation, hence, their fitness and physiology fluctuates with environmental temperature (Mordecai et al., 2019; Paaijmans et al., 2013). Moreover, temperature-dependent food resource availability in aquatic habitats (Fillinger et al., 2009; Zettle et al., 2022) determines fitness traits during immature stages of development and fitness benefits carried-over to the adult stage (Evans et al., 2020; McCormick & Gagliano, 2008). High temperature stress, in paper I and II, shortened larval duration resulting in accelerated adult emergence of all the four species, irrespective of larval resource level (Figure 3A-D), as previously reported (Christiansen-Jucht et al., 2014, 2015; Ezeakacha & Yee, 2019; Kirby & Lindsay, 2009; Lyons et al., 2013). Larval diet provides somatic energy for basal sustenance, and to cope with temperature stress since metabolism scales linearly with temperature (Briegel & Timmermann, 2001; Klepsatel et al., 2019). Therefore, meagre food resources negatively affect fitness traits of immature stages of mosquitoes. This is associated with extended larval duration to allow for sufficient biomass accumulation to support pupation, as observed in extended time to adult emergence when food resource is restricted (Figure 3A-D) (Briegel et al., 2001; Carvajal-Lago et al., 2021; Couret et al., 2014; Evans et al., 2021; Huxley et al.,2021, 2022). However, despite an extended larval phagophase duration, under limited food resources, *Anopheles* species (Figure 3B) did not transition to pupae but *Ae. aegypti* (Figure 3A) did, indicating disparities among mosquito genera in coping with temperature stress and food resource limitation (paper I). These disparities corroborate previous reports, albeit under constant temperatures, on species differences in coping strategies to starvation and temperature stress (Ayala et al., 2014; Bennett et al., 2021; Briegel, 1990a, 1990b), which could impact vector population dynamics.

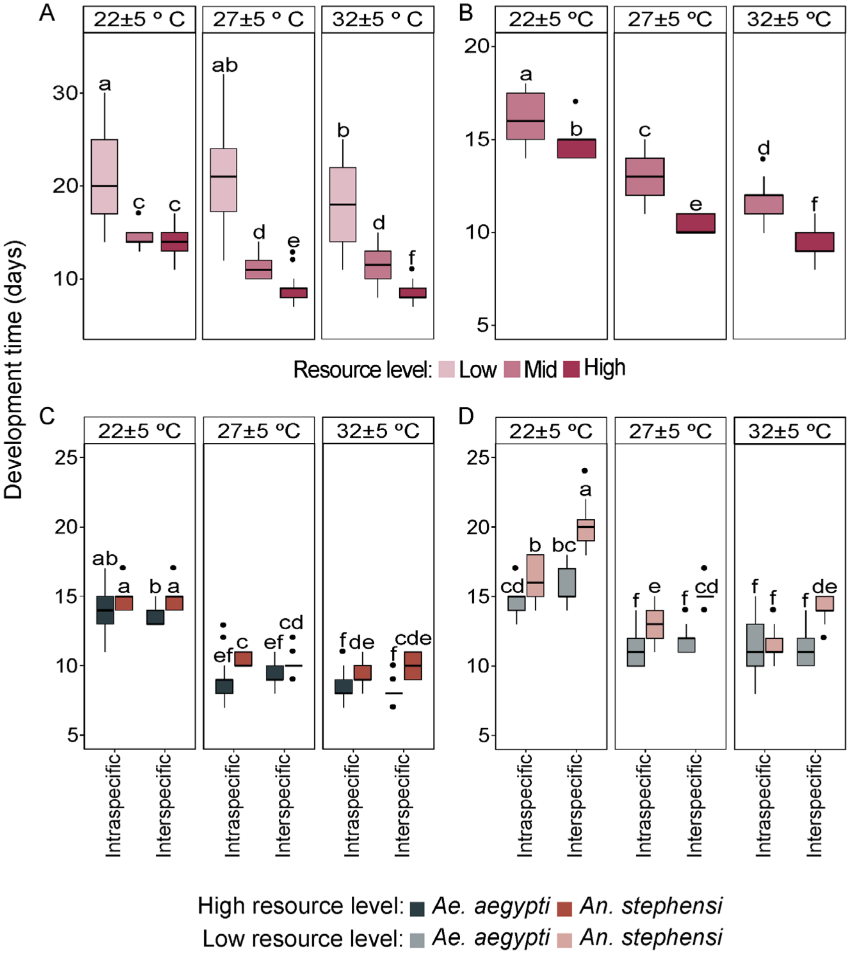


Figure 3. Temperature and larval resource level interact to influence the immature stages of mosquitoes. Development time from egg hatching to adult emergence of (A) *Aedes aegypti*, (B) *Anopheles stephensi*. Lowercase letters, from pair-wise comparisons of emmeans, indicate significant interactions of temperature and resource level on the development time (P<0.05; mixed-effect Cox regression analysis) (Jacob et al., 2026). Development time of sympatric mosquitoes: (C) competition between *Ae. aegypti* and *An. stephensi* under high larval resource level, (D) competition between *Ae. aegypti* and *An. stephensi* under low larval resource level. The intraspecific data sets are from Jacob et al. (2026). Significant interactions of temperature, resource level and mode of competition on development time are indicated by lower case letters (P<0.05; mixed-effect Cox regression analysis) (paper II).

5.2 Carry-over effects of temperature stress and larval resource level on adults

5.2.1 Size and survival

The observed trade-off between accelerated adult emergence and adult size, at high temperature, in all the four species in paper I and II (Figure 4A-F) corroborated previous studies (Evans et al., 2021; Ezeakacha & Yee, 2019; Kirby & Lindsay, 2009; Lyons et al., 2013). Increased metabolic demand to cope with high temperature stress (Djawdan et al., 1998; Klepsatel et al., 2019) may reduce nutrient allocation for structural biomass since acquired nutrition is used for basal sustenance during larval development, as reflected in reduced size of emerging adults under stressful conditions. Moreover, in papers I and II, effects of temperature stress on adult size are enhanced by larval food quantity (Huxley et al., 2021, 2022), in which under restricted resource regime a trade-off between larval duration and structural biomass is reflected in the reduced size of emerging adults (Figure 4A, B).

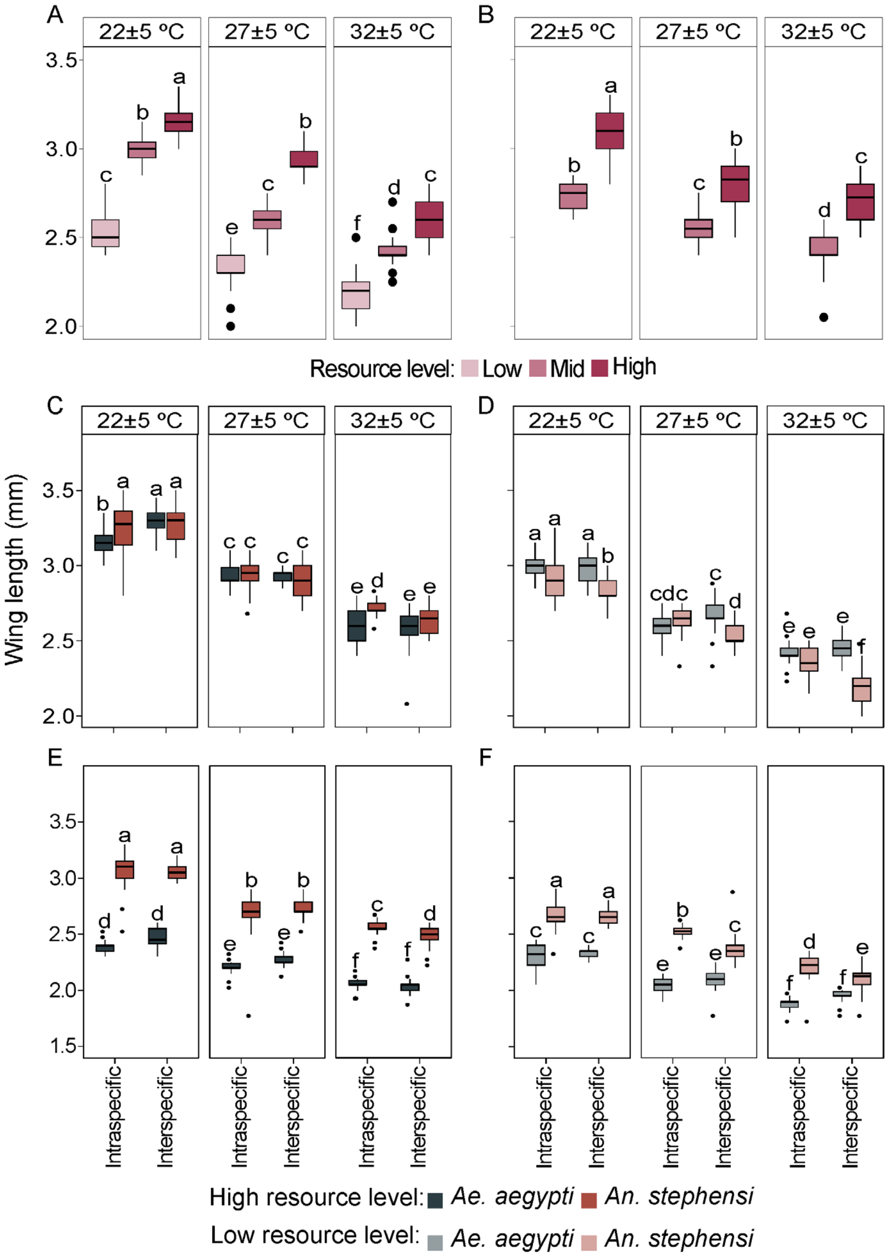


Figure 4. Temperature and larval resource level interact to influence adult size of mosquitoes. The wing length of (A) *Ae. aegypti*, (B) *An. stephensi*. Lowercase letters, from pair-wise comparisons of emmeans, indicate significant interactions of temperature and diet level on the size for each species (P<0.05; mixed-effect analysis of variance)

(Jacob et al., 2026). Size of sympatric female (C, D) and male (E, F) *Ae. aegypti* and *An. stephensi*. The intraspecific data sets are from Jacob et al. (2026). Significant interactions of temperature, resource level and mode of competition on adult size are indicated by lower case letters (P<0.05; mixed-effect analysis of variance) (paper II).

Increase in temperature stress reduced the survival of starved adults of all species in paper I and II (Figure 5A, B; Figure 7A-D). During starvation, insects rely on metabolic reserves accumulated during larval development (Arrese & Soulages, 2010). Extended larval phagophase duration at low temperature allows for accumulation of sufficient metabolic reserves to sustain longer adult survival during starvation, while a shortened larval duration at high temperature negatively impacts reserve accumulation (Briegel et al., 2001). Moreover, metabolic rate scales linearly with temperature leading to accelerated catabolism of stored reserves at high temperature, which negatively affects adult survival (Klepsatel et al., 2019), and accelerated physiological senescence in mosquitoes at high temperature stress diminishes longevity (Barr et al., 2024, 2025; Martin et al., 2025). Negative effect of temperature stress on survival in papers I and II was accentuated by limited food resources during larval development (Evans et al., 2021; Huxley et al., 2021, 2022) (Figure 5A, B; Figure 7A-D). However, at high temperature stress, longevity of the four species in paper I was not dependent on larval resource level, indicating possible mechanisms for coping with stress (Ayala et al., 2014; Bennett et al., 2021; Briegel, 1990a, 1990b; Dennington et al., 2024). In agreement with the size, temperature and fitness rule (Kingsolver & Huey, 2008.), larger insects survived longer, although a diminished effect of size on survival at high temperature in paper I was noted (Figure 5A, B). These findings corroborate the energy tolerance to stress concept, whereby larger insects have a higher metabolic demand for basal sustenance (Sokolova, 2013). Although high temperature stress results in smaller adults with a diminished lifespan, accelerated adult emergence may result in stable mosquito populations (Ruiz-Herrera, 2017), an outcome significant to disease transmission dynamics.

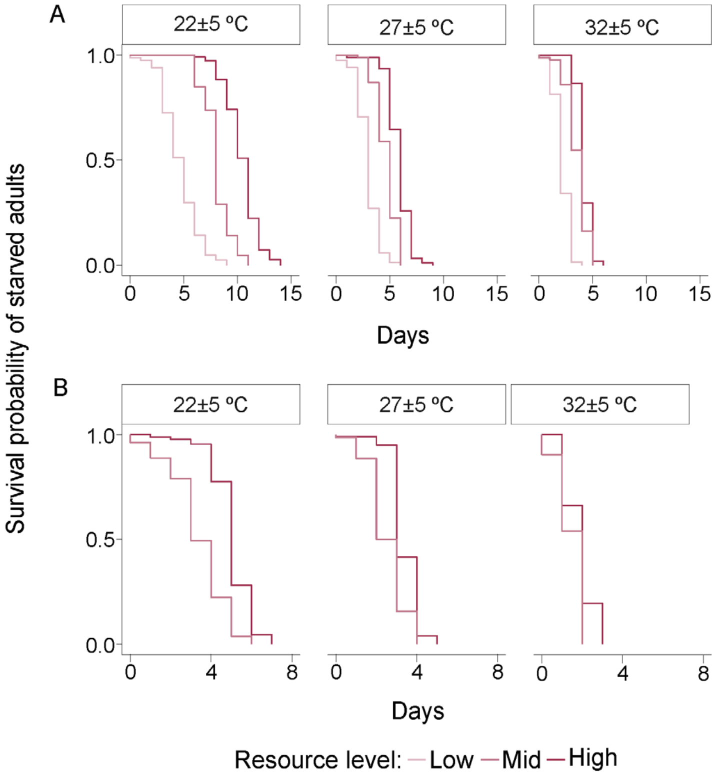


Figure 5. Temperature and larval resource level interact to influence probability of adult survival of (A) *Ae. aegypti* and (B) *An. stephensi* (Jacob et al., 2026).

5.2.2 Metabolic reserves

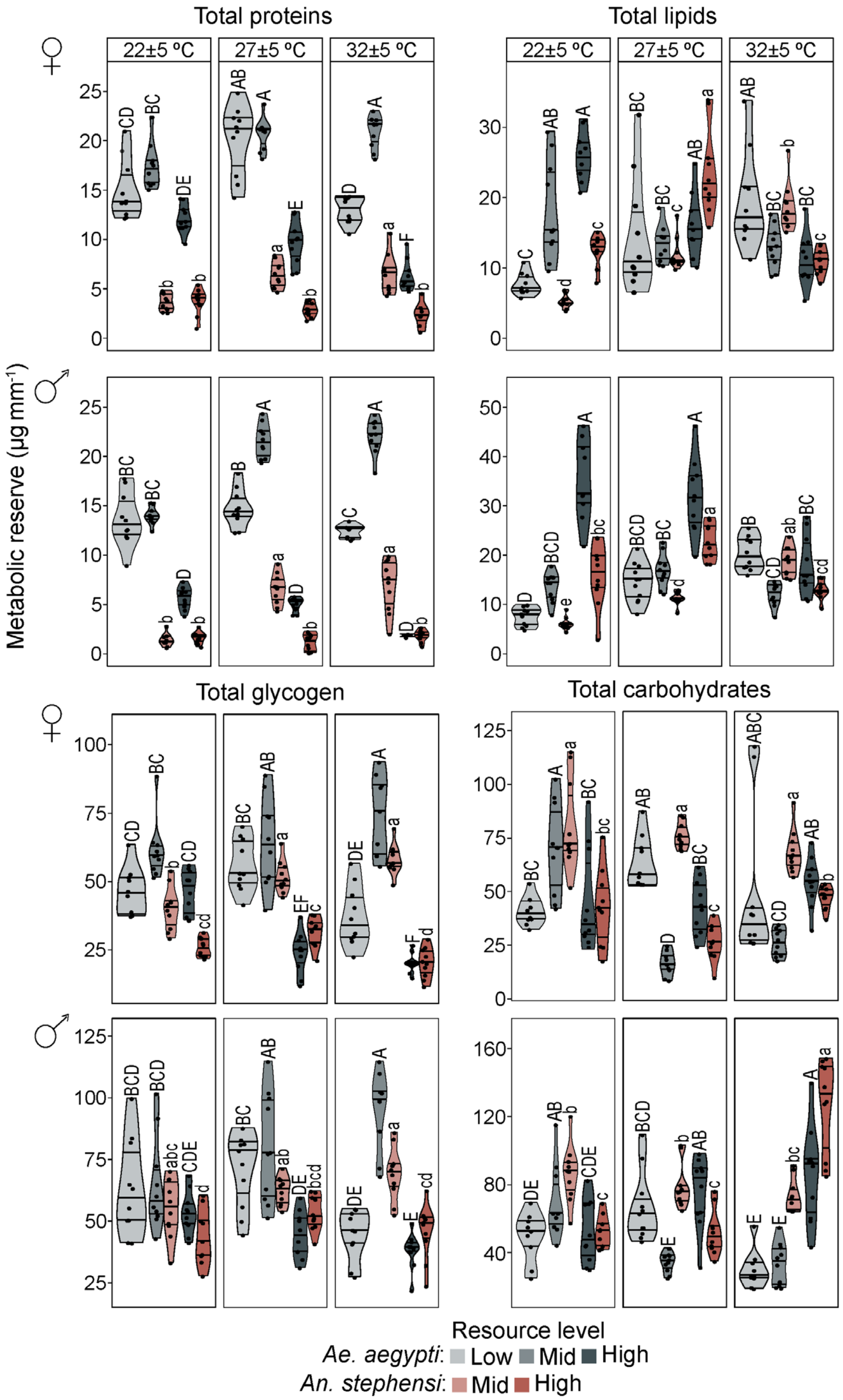
Metabolic reserves accumulated during larval development provide somatic fuel for survival and flight in teneral adults (Briegel, 1990b, 1990a; Briegel et al., 2001; Timmermann & Briegel, 1999). Temperature in aquatic habitats determines larval feeding duration, feeding rate and the content of nutrients reserved, while food quantity provides biomass (Briegel et al., 2001; Timmermann & Briegel, 1999). In paper I, the observed disparities in protein, glycogen, lipids and carbohydrates content between *Ae. aegypti* and anophelines corroborates previous studies on how the two genera accumulate reserves under different conditions during aquatic development (Briegel, 1990a, 1990b; Briegel et al., 1979, 2001). For instance, *Ae. aegypti* earned the nickname ‘obese’ mosquito due to its efficiency in reserves accumulation (Van Handel, 1965). In this study, varying temperature and resource regimes differentially determined metabolic content carried over to teneral adults from larval stages, with a higher protein content in *Ae. aegypti* compared *Anopheles* species (paper I; Figure 6) which likely contributed to longer survival of the former compared to later species (Figure 5A, B; Figure 7A-D). Despite being a structural component, protein may provide metabolic fuel during adverse conditions. For instance, insects use proline for flight energy (Arrese & Soulages, 2010; Scaraffia & Wells, 2003).

Disparities in long term reserves, lipid and glycogen, at low vs high temperature (Figure 6) may have influenced the survival of starved adults in papers I and II maintained under similar conditions during larval development, in which higher contents led to increased longevity (Figure 5A, B; Figure 7A-D). The observed differences in total carbohydrates among species across different temperature and resource regimes (paper 1) may be due to disparities on the phosphorylase activity on glycogen to provide glucose needed for synthesis of chitin, and energy for transition of pupae to adults (Arrese & Soulages, 2010; Steele, 1982). Taken together, metabolic reserves carried over from larval stages to teneral adults are key to mosquito survival under varying biotic and abiotic stress, a trait significant to vectorial capacity (Smith et al., 2012). In addition, metabolic reserves influence subsequent host preference of mosquitoes as they seek to replenish their energy (Hancock & Foster, 1997).

5.2.3 Feeding propensity

Teneral mosquitoes primarily seek to replenish their metabolic reserves from sugar rich sources (Barredo & DeGennaro, 2020; Foster, 1995). However, biotic stress in larval habitats such as poor food resources may render malnourished teneral females thirsty for blood as a source of energy for survival, as well as ovarian maturation (Briegel & Hörler, 1993). In paper I, high temperature stress experienced during larval development differentially increased feeding propensity on sugar (honey) among the four species while a restricted larval food resource enhanced the effects temperature stress on feeding, reflecting a reliance of carbohydrates to replenish metabolic energy during stress.

A high blood feeding propensity of teneral adults in paper I, reared under a high larval resource level likely reflects ovarian maturation due to high lipid content carried over during larval development (paper I; Figure 6) (Dittmer et al., 2019). However, blood feeding by teneral adults from high temperature and low larval resource level, although at a low propensity (paper I), likely indicates use of this meal for energy since under similar adverse conditions, mosquitoes emerged with low teneral reserves (paper 1; Figure 6). Disparities on how temperature stress influences blood feeding among mosquito species as they seek to replenish metabolic fuel is reflected on varying feeding propensity, in which *Ae. aegypti*, *An. stephensi* and *An. coluzzii* fed more at intermediate temperature while *An. arabiensis* fed more at low temperature (paper I). Overall, the impact of biotic and abiotic stress on feeding may have significant implications for disease transmission. For instance, multiple bouts of blood feeding increases vector-human interactions as mosquitoes seek the proteinacious meal for energy and reproduction (Briegel & Hörler, 1993; Carvajal-Lago et al., 2021).


Total proteins
Total lipids
Total glycogen
Total carbohydrates
♀
♂
22±5 ºC
27±5 ºC
32±5 ºC
Metabolic reserve (µg mm⁻¹)
0
5
10
15
20
25
30
40
50
75
100
120
125
160
CD
BC
b
DE
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AB
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CDE
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BCD
E
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bc
A
a
Resource level
Ae. aegypti: Low Mid High
An. stephensi: Mid High

Figure 6. Temperature and larval resource level interact to influence the teneral metabolic reserves of mosquitoes. Mean content of metabolic macronutrients, adjusted by mean wing size, in male and female *Ae. aegypti* and *An. stephensi*. Upper- and lower-case letters, from pair-wise comparisons of emmeans, indicate significant interactions between temperature and resource level on metabolic reserves (P<0.05; mixed-effect analysis of variance) (female data is from Jacob et al., 2026).

5.3 Temperature stress and larval resource level affect competition outcomes of sympatric mosquito species

5.3.1 Development time

Larvae of sympatric species compete for food resources in aquatic habitats to cater for somatic metabolic demand for growth and survival across different temperature gradients (Couret et al., 2014; Evans et al., 2021; Kirby & Lindsay, 2009). In paper II, *Ae. aegypti* developed faster in the presence or absence of *An. stephensi* (Figure 3C, D), while under restricted food resources, the former species slowed the development of the later species (Figure 3D). This corroborates previous studies on *Aedes* species ability to suppress growth of heterospecifics in shared habitats likely due to efficient feeding habits as a shredder, production of retardant factors and starvation tolerance (Barrera, 1996; Merritt et al., 1992; Moore & Whitacre, 1972; Padmanabha et al., 2011).

5.3.2 Adult size and survival

Despite accelerated development of *Ae. aegypti* in paper II, females were similar or larger in size compared to *An. stephensi*, when reared under high or limited larval food resources, respectively, (Figure 4C, D) indicating starvation tolerance and efficient conversion of food to structural biomass by *Aedes* species (Barrera, 1996; Briegel, 1990a, 1990b; Van Handel, 1965). However, this was not the case for males as they emerged smaller compared to *An. stephensi* (Figure 4E, F), likely reflecting a sex divergence in coping with biotic and abiotic stress. The negative effect of competition on the size of male *An. stephensi* in the presence of *Ae. aegypti* at high temperature stress and limited food resources (Figure 4F) supports the conclusion that *Ae. aegypti* is a stronger competitor for food resources (Evans et al., 2021). Although mosquitoes segregate habitats to cope with competition (Leisnham et al., 2014), slow development (Figure 3D) allowing for a release from competition of less competitive by dominant species will produce large insects (Figure 4C, E, F) which in the long run influences fecundity and pathogen transmission (Barreaux et al., 2018; Yan et al., 2021).

Differences in temperature preference by mosquitoes was reflected in increased longevity of *An. coluzzii*, at low temperature, when reared with *An. arabiensis* in paper II. This corroborates seasonal temperature separation of the two species in the field, whereby *An. arabiensis* is abundant during dry warm conditions while *An. coluzzii* dominates cool wet seasons (Mala et al., 2011). The extended survival of female (Figure 7A, B) and male (Figure 7C, D) *Ae. aegypti* compared to *An. stephensi* contradicts the size, temperature and fitness rule (Kingsolver & Huey, 2008), because males of the later species were larger compared to the former species (Figure 4E, F), while females of *An. stephensi* from the high larval resource diet were of similar size to *Ae. aegypti* (Figure 4C). This supports the conclusion that *Aedes* are more efficient than *Anopheles* species in amassing metabolic reserves during larval development (paper I; Figure 6), as well as starvation tolerance during adverse conditions (Barrera, 1996; Briegel, 1990a, 1990b; Van Handel, 1965). Taken together, the observed effects of temperature and larval resource level on asymmetric competition broadens our understanding of how environmental factors shape fitness traits relevant to population growth dynamics and vectorial capacity of sympatric mosquito species.

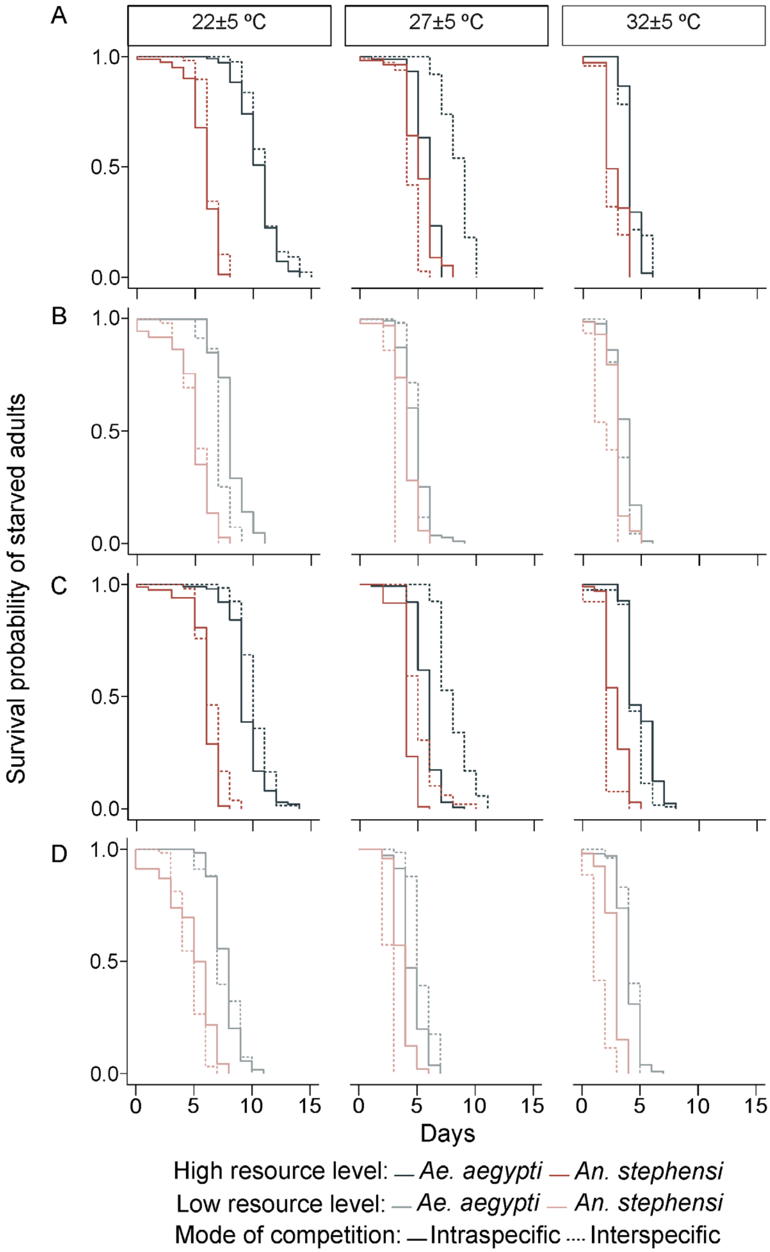

Figure 7. Temperature and larval diet level interact to influence the survival probability of sympatric mosquitoes. Female (A, B) and male (C, D) *Ae. aegypti* and *An.* in response to inter- and intra- specific competition (paper II). The intraspecific data sets are from Jacob et al. (2026).

5.4 Temperature, age and their interaction affect dynamic feeding of *Anopheles coluzzii*

The chronological balance in carbon and nitrogen intake by mosquitoes from sugar and blood meals is dependent on somatic metabolic fuel requirements and reproduction (Foster, 1995; Hill & Ignell, 2021; Takken & Knols, 1999). Biotic and abiotic stress during larval development influences metabolic reserves carried-over to adults, and subsequent feeding by teneral adults on sugar and blood, as demonstrated in paper I and by (Foster, 1995; Takken & Knols, 1999). After building up metabolic fuel reserves, sugar foraging diminishes in older females (Foster & Takken, 2004; Hill & Ignell, 2021). For instance, in paper III, at constant temperature and high diurnal fluctuating temperature stress, more teneral compared to older females fed and ingested a high volume of honey. However, at low fluctuating temperature stress, propensity to feed on honey was low (paper III), indicating a diminished requirement for sugar due to high metabolic reserves carried-over from larval stages (paper I), as well as low metabolic rate (Klepsatel et al., 2019).

Mosquito blood feeding propensity increases with ageing following ovarian maturation due to sufficient lipid reserves in the ovaries (Clements, 1992; Dhadialla & Raikhel, 1990; Foster, 1995). Under lower fluctuating temperature conditions, a high blood feeding propensity increased with insect maturation (paper III). However, a lack of age-dependent feeding at high fluctuating temperature (paper III), likely reflects a reliance of blood to supplement energy requirements during stress.

Urine and caterpillar hemolymph have been reported to supplement energy requirements, providing survival and reproductive benefits to mosquitoes (Dawit et al., 2022; George et al., 2014; Martel et al., 2011). Similarly, the increased proportion of young females ingesting urine at high fluctuating temperature stress indicates a reliance on this nitrogen-rich resource by mosquitoes to replenish their metabolic energy (paper III). On the other hand, high levels of feeding by older females at constant and high fluctuating temperature (paper III) likely reflects allocation of urine for reproductive output (Dawit et al., 2022). Overall, age-dependent feeding by *An. coluzzii* provides insight into how environmental stress and insect physiology shapes nutrient acquisition, which has significant repercussions in disease transmission dynamics.

6. Conclusion and future perspectives

Overall, biotic and abiotic stress determines immature life history traits and fitness benefits carried over to adult mosquitoes, which influences their behaviour in a species-dependent manner. Varying effects of temperature and larval resource stress, among mosquito species, during aquatic development on adult metabolic reserves, survival and feeding propensity were observed. These findings provide a novel comparative assessment of how environmental stress may shape vector population dynamics and disease transmission. In addition, the asymmetric competition of sympatric invasive species during stressful conditions provides a deeper insight into how environmental stress might structure vector density, fitness, as well as vectorial capacity. The novel temperature and age effects on dynamic feeding by *An. coluzzii* provide insight into how ecologically relevant temperature conditions affect nourishment from different meals.

Based on the findings from this study, the following future perspectives are proposed:

- Predictive models on effects of temperature on vector population dynamics should incorporate how feeding varies with temperature stress, to provide a holistic understanding of disease transmission dynamics.
- Findings from this study provide an overview of intragenerational outcomes of temperature and food resource stress on mosquitoes. Do biotic and abiotic stress have similar intergenerational impact on different mosquito species?
- Temperature affects physiological senescence of mosquitoes, therefore, is the blood ingested by teneral adults reared under varying temperature regimes allocated for metabolic energy or

reproduction? Based on this, how does temperature stress affect reproduction and by extension, disease transmission?

- Do wild mosquito populations from different altitudinal gradients display similar fitness, behaviour and competitive outcomes as laboratory reared species?

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Popular science summary

Disease vectoring mosquitoes are the most dangerous animals on earth due to morbidities and mortalities associated with their infectious bites. For example, *Anopheles gambiae* mosquitoes transmit malaria which kills a child every 30 seconds while *Aedes* borne viruses such as dengue, chikungunya and yellow fever afflict millions globally. Despite the use of different arsenals aimed at vector control, eradication of mosquito borne diseases is faced a by plethora of challenges, some of which are exacerbated by global warming. For instance, temperature-dependent expansion in geographical range distribution of different mosquito species poses a burden in control efforts.

As ectotherms, mosquitoes cannot regulate their homeostatic temperature. Therefore, temperature stress in aquatic habitats regulates egg hatchability, development rate, larval duration, as well as feeding and metabolic rates. In addition, food resource abundance in aquatic habitats such as microorganisms, microbial breakdown products and pollen, required by larvae for somatic energy and to cope with temperature stress, fluctuates with environmental temperature. Therefore, fitness benefits carried-over to the adult stage including size, survival, metabolic reserves and feeding fluctuate with environmental temperature.

While effects of constant temperatures and larval food quantity on various mosquito fitness traits have been extensively evaluated, how interactions of naturalistic fluctuating temperature and food resources impact mosquito fitness, competition and behaviour remain scant. During their lifetime different mosquito species experience a plethora of temperature fluctuations in their habitats. Therefore, fluctuating environmental conditions provide a better understanding of how biotic and abiotic stress impacts disease vectors.

Diurnal fluctuating temperature and varying larval resource levels were used in this study to comparatively elucidate how the two environmental factors interact to impact immature fitness traits and subsequent carry-over benefits to adults of *Ae. aegypti*, *An. stephensi*, *An. coluzzii* and *An. arabiensis*. Interaction of fluctuating temperature and larval resource level affected immature and adult life history traits, as well as fitness benefits carried-over from larval to adults in a genera-dependent manner. In addition, the two environmental factors shaped asymmetric competition for food by larvae of different sympatric species; *Ae. aegypti* and *An. stephensi*, as well as *An. coluzzii* and *An. arabiensis*, while the observed effects of temperature stress on age-dependent feeding by *An. coluzzii* on honey, blood and urine renders support on how temperature impacts nutritional status and ensuing behaviour of mosquitoes. Differential response of mosquitoes to biotic and abiotic stress provides insight on how population growth rate, community structure and disease transmission dynamics may vary, in view of global warming.

Populärvetenskaplig sammanfattning

Sjukdomsspridande myggor är de farligaste djuren på jorden på grund av de sjukdomar och dödsfall som är förknippademed deras smittsammabett. Till exempel sprider *Anopheles gambiae*-myggor malaria, vilket dödar ett barn var 30:e sekund, medan *medan virus som sprids av Aedes-myggor* såsom dengue, chikungunya och gula febern drabbar miljontals människor globalt. Trots användning av olika strategier för vektorkontroll, står utrotning av myggburna sjukdomar inför en mängd utmaningar, varav vissa förvärras av den globala uppvärmningen. Till exempel innebär temperaturberoende utvidgningav olika myggarters geografiska utbredning en ytterligare belastning på kontrollinsatser.

Som ektoterma kan myggor inte reglera sin kroppstemperatur. Därför påverkartemperaturstress i akvatiska habitat deras äggkläckning, utvecklingshastighet, larvstadiets längd samt födo- och ämnesomsättningshastigheter. Dessutom varierar tillgången på födoresurser i akvatiska habitat, såsom mikroorganismer, mikrobiella nedbrytningsprodukter och pollen- som larver behöver för somatisk energi och för att hantera temperaturstress- med omgivningstemperaturen. Därför varierar även de fitnessfördelar som förs vidare till vuxenstadiet, inklusive storlek, överlevnad, metabola reserver och födointag, med den omgivande temperaturen.

Även om effekterna av konstanta temperaturer och larval födotillgång på olika fitnessrelaterade egenskaper hos myggor har studerats, är kunskapen begränsad om hur samspelet mellan naturligt fluktuerande temperaturer och födoresurser påverkar myggors fitness, konkurrens och beteende. Under sin livstid utsätts olika myggarter för en mängd temperaturvariationeri sina habitat. Därför ger fluktuerandemiljöförhållanden en bättre förståelse för hur biotisk och abiotisk stressfaktorer påverkar sjukdomsvektorer.

I denna studie användes dygnsvisa temperaturvariationer och varierande nivåer av larval födotillgångför att undersöka hur de två miljöfaktorerna samverkar för att påverka fitnessrelaterade egenskaper hos omogna stadier samt efterföljande effekter i vuxenstadiet hos *Ae. aegypti*, *An. stephensi*, *An. coluzzii* och *An. arabiensis*. Samspelet mellan fluktuerande temperatur och larval födotillgångpåverkade livshistorieegenskaper hos både omogna och vuxna stadier, liksom de fitnessfördelar som fördes vidare från larver till vuxna på ett släktberoende sätt. Dessutom formade de två miljöfaktorer asymmetrisk konkurrens om föda mellan larver av olika samexisterandearter; *Ae. aegypti* och *An. stephensi*, samt *An. coluzzii* och *An. arabiensis*. Vidare ger de observerade effekterna av temperaturstress på åldersberoende födointaghos *An. coluzzii*- av honung, blod och urin- stöd för hur temperatur påverkar myggors näringsstatus och därmed deras beteendet. Differentierad respons hos myggor på biotisk och abiotisk stress ger insikt i hur populationstillväxt, samhällsstruktur och sjukdomsöverföringsdynamik kan variera med tanke på den globala uppvärmningen.

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To my family, I am indebted to you for all your endearing support during this journey.

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Parasites & Vectors

RESEARCH **Open Access**

Diurnal fluctuating temperature and larval resource level interact to influence the life history and behaviour of disease-transmitting mosquitoes

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Abstract

Background The global rise in temperature has seen a geographical range increase in mosquito populations and disease transmission. Temperature affects life history traits of mosquitoes, and hence, population dynamics and vectorial capacity. In addition, food resource abundance, required for biomass and somatic energy during mosquito larval development is dependent on temperature. How the interaction between temperature and food resources affects the life history traits of aquatic stages, and subsequent carry-over effects to the adult stage, under simulated natural conditions, remains underexplored.

Methods A comparative assessment of the interactive effect of diurnal fluctuating temperature and resource level during larval development on life history traits of the yellow fever mosquito, *Aedes aegypti*, and three malaria vectors, *Anopheles stephensi*, *Anopheles coluzzii* and *Anopheles arabiensis* was conducted. Moreover, carry-over effects on teneral adults including, metabolic reserves and propensity to feed were evaluated on the four species under similar abiotic conditions. A total of 2700 larvae of each species were reared under three fluctuating temperature regimes, and maintained on different resource levels. A mixed-effects Cox regression model was used to determine effects of the two environmental factors on the time to adult emergence, and adult survival. Generalised linear mixed-effect model with a binomial error structure was used to elucidate effects of abiotic stress on feeding, whereas linear-mixed effects analysis of variance, was used to estimate the effects of temperature and resource level on adult size and metabolic reserves. Aligned Rank Transform analysis of variance was used to determine effects of abiotic stress on level of feeding. Correlation between size and survival of starved adults was determined by multivariate analysis using Spearman's rank correlation and linear regression.

Results Time to adult emergence shortened with increasing temperature and resource level. Accelerated adult emergence was associated with reduced adult size and survival at high temperature, in a resource-dependent manner. Metabolic macronutrient reserves carried over into teneral adults were differentially regulated by temperature and larval resource level, in a species dependent manner. Teneral females engaged in feeding on honey or blood depending on the two abiotic stressors, and species.

Conclusions Temperature and resource level during larval development differentially affects life history traits of disease-transmitting mosquitoes, which have ramifications on population size, as well as disease transmission dynamics.

Keywords Carry, Over effects, Temperature, And resource, Dependent life history traits, Mosquitoes

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Background

Vector-borne diseases are a key global health challenge, exacerbated by climate change as a consequence of anthropogenic activities [1–3]. As ectotherms, the life history traits, physiology and behaviour of mosquitoes are sensitive to changes in temperature, which ultimately determine their geographical distribution and vectorial capacity [4–15]. However, previous studies do not fully reflect the effect of temperature on mosquito life history and physiology, as most were conducted under constant temperatures. Therefore, to better predict disease spread, it is important to study how varying temperatures will affect vector population dynamics in the face of climate change [16, 17]. Temperature conditions may, likewise, affect larval food availability in breeding habitats, including, *e.g.*, microorganisms and plant detritus [18, 32–34] by regulating the abundance of ectothermic microorganisms [63]. Subsequently, this will indirectly regulate fitness parameters of mosquito larvae and adults [19]. In light of global warming, understanding the response of mosquitoes, and their associated food sources, to fluctuating temperatures may be critical for generating accurate future geographical distribution models [18], as well as estimating vector availability and human biting rate in vectorial capacity models of malaria and dengue transmission [13, 20].

Available data on the effect of diurnal temperature fluctuation on mosquito life history traits emphasise that this effect is non-linear, unimodal and restricted within temperature safety margins, defined as critical temperature maxima and minima within which optimal fitness is displayed [13, 14, 16, 20]. For instance, vital life history traits for malaria and dengue vectors are optimal within 23 °C—29 °C, below or above which the development rate, survival, fecundity, biting rate and pathogen transmission decline [13, 16, 20]. Mosquito temperature safety margins are dependent on habitat temperature ranges, with safety margins being more constricted for tropical species and during summer for temperate species, with the latter broadening the margin during cooler months [21, 22]. Moreover, temperature fluctuations within an ecological zone may influence mosquito life history traits. For instance, the aquatic stages of the malaria vector *Anopheles arabiensis* is more tolerant to higher temperatures compared with *Anopheles funestus* and the sympatric species, *Anopheles gambiae* sensu stricto [23]. At high average daily temperatures, saltatory diurnal temperature changes within the critical temperature range reduced the development rate, survival and increased the length of the gonotrophic cycle, and vectorial capacity of the malaria vectors, *An. gambiae s. s.* and *Anopheles stephensi*, as well as the dengue vector, *Aedes aegypti* [24–27].

Studies examining the effects of temperature on mosquito physiology and behaviour are limited. However, existing reports suggest that higher constant daytime temperatures are negatively correlated with metabolic reserves, adult size and blood-feeding behaviour of mosquitoes [8, 28]. As such, cross-species analysis of the effects of fluctuating temperature on the life history parameters of larvae and the ensuing carry-over effects, *i.e.*, the irreversible larval fitness advantages transferred to the adult stage [29] should be elucidated. This may provide a much-needed holistic understanding of the differential impact of global warming on major malaria and dengue vectors.

Food availability affects mosquito fitness because metabolic rate scales with temperature [64]. Therefore, the teneral adult somatic energy requirements across different temperature gradients is limited by larval food abundance [19, 30, 31]. Larvae from nutrient-rich habitats, irrespective of temperature stress, have a 'silver spoon' advantage, *i.e.,* a better start [29], and produce adults with increased fitness, immunity and metabolic reserves, while those from resource-poor habitats have reduced fitness and reserves [4, 19, 29, 35–40].

Under natural conditions, how the interaction between temperature and food resources shape fitness and, by extension, the vectorial capacity of mosquitoes, remains elusive. In this study, the interactive effect of dynamic temperature and varying resource levels, on mosquito development time, and the carry-over effects on adult life history traits, physiology and behaviour were evaluated. To gain insight into the variability of this effect, a comparative analysis was made among *Ae. aegypti* and three major malaria vectors, *An. stephensi*, *Anopheles coluzzii* and *An. arabiensis.* Our findings provide insight into how the interaction of environmental factors may shape the fitness, and potentially the vectorial capacity, of mosquito vectors in view of climate change.

Methods

General colony maintenance

Long standing laboratory colonies of *Aedes aegypti* (Rockefeller), *An. stephensi* (type-form, SDA-500), *An. coluzzii* (G3) and *An. arabiensis* (Dongola) were reared at a 12 h: 12 h light: dark photoperiod, 25 ± 2 °C and 65 ± 5% relative humidity. Adults were provided 10% sucrose *ad libitum*, and mature females offered defibrinated sheep blood (Håtunalab AB, Bro, Sweden) via membrane feeding (Hemotek Ltd, Blackburn, UK). Ovicups (30 ml; Nolato Hertila AB, Åstorp, Sweden) lined with filter paper (90 mm; Whatman®, Thermo Fischer Scientific, Gothenburg, Sweden) and half filled with distilled water were provided as an oviposition substrate to gravid females for egg deposition. Eggs were hatched in plastic trays (7 cm × 18 cm × 20 cm), containing 1 L of distilled water. Upon emergence, larvae were maintained on ca ~0.75 mg larva day^{-1} of Tetramin® fish food (Tetra GmbH, Melle, Germany) until pupation. Pupae were collected into ovicups (30 ml; Nolato Hertila AB), which were placed in Bugdorm cages (30 cm × 30 cm × 30 cm; MegaView Science Co., Ltd, Taichung, Taiwan), and emerging adults maintained as described above.

Experimental design

To evaluate how ambient temperature and resource restriction interactively affect fitness and feeding behaviour of disease vectors, a 3 × 3 × 4 factorial design, including three resource regimes, three fluctuating diurnal temperature ranges, and four mosquito species, was used (Additional file 1: Supplementary Fig. S1). Experimental chambers (IPP750ecoplus; Memmert GmbH, Büchenbach, Germany) were programmed to either of the three temperature regimes, 22 ± 5 °C, 27 ± 5 °C and 32 ± 5 °C with a gradual increase and decrease in temperature during photophase and scotophase, respectively, reflecting the natural change on the basis of meteorological data (AccuWeather; Kilifi, Kenya). The low temperature was fluctuated at a range of 17–27 °C around the mean of 22 °C, intermediate temperature at 22–32 °C around a mean of 27 °C and high temperature at 27–37 °C around a mean of 32 °C (Additional file 1: Supplementary Fig. S2). The temperature regimes were selected on the basis of the geographical range distributions of the four mosquito species [41–44], reflecting both cooler and warmer climates. A photoperiod of 12 h: 12 h light: dark was used, and a 30 min gradual transition, at 30% light intensity, between photoperiods was adopted to simulate sunrise and sunset. Relative humidity in the experimental chambers was not regulated and it ranged from 90 to 100%. Data loggers (Gemini Data Loggers Ltd, Chichester, UK) were used to monitor temperature and humidity in the climatic chambers throughout the experimental period.

For *Ae. aegypti*, a high resource level of Tetramin® fish food (1 mg larva^{-1} day^{-1}) was selected on the basis of previous studies showing this to be the amount of food supporting the highest larval survival [19]. To measure the effect of resource limitation, the high resource level was scaled down by a factor of $10^{0.5}$ and 10^{1} to obtain a 'mid'- and low-resource level of 0.32 mg larva^{-1} day^{-1} and 0.1 mg larva^{-1} day^{-1}, respectively. This resource regime was equally adopted for the maintenance of *An. stephensi* since the two species are known to share breeding habitats [45]. For *An. coluzzii* and *An. arabiensis*, a high resource level of 0.75 mg larva^{-1} day^{-1} was adopted from amounts used in routine laboratory colony maintenance of these species [46]. This amount was scaled down using similar factors as in *Ae. aegypti* to obtain a mid- and low-resource level of 0.24 mg larva^{-1} day^{-1}and 0.075 mg larva^{-1} day^{-1}, respectively.

To begin the experiments, eggs obtained from laboratory colonies of the four species were hatched in distilled water that was previously incubated in the three experimental chambers for 24 h. Cohorts of 50 first-instar larvae were transferred into 1 L distilled water in plastic trays (7 cm × 18 cm × 20 cm). For each species, this was replicated six times resulting in 6 × 3 temperature ranges × 3 resource levels = 54 experimental units, and a total number of 10,800 mosquitoes. Owing to space limitation in experimental chambers, the effect of abiotic stress on all species was not evaluated at the same time. Therefore, the effect each resource level on fitness traits across temperature regimes was evaluated per species, at a time.

Fitness traits

Daily larval mortality was monitored for each treatment, and the amount of larval food adjusted so as to maintain a constant resource supply (mg larva^{-1} day^{-1}) throughout the experiment. To manage resource quantity and prevent detrimental microbial growth, larval trays were cleaned and the water changed daily. For each replicate per treatment, pupae were collected and pulled into ovicups as per the date of pupation. The ovicups were placed in Bugdorm cages (MegaView Science) and, upon emergence, the adults were maintained on distilled water throughout their life span in the experimental chambers. The development time of mosquitoes surviving to adult stage was determined as the time from egg hatching to adult emergence. Adults were grouped as per the date of emergence to account for their survival. Survival of the starved adults was used to evaluate whether metabolic reserves carried over from immature stages contributed to resilience of the four species when subjected to different temperature and larval resource regimes. The wing length (mm) from five females per replicate in each treatment was used as a proxy for adult size [47]. Length of the left wing was measured from the distal end of the alula up to the tip, excluding the fringe, using a dissecting microscope fitted with an ocular micrometer. To establish whether the survival of starved adults was dependent on size, the correlation between average size and survival, *i.e.*, time to death (in days), for each temperature and resource level, was determined.

Metabolic macronutrient energy reserves

Metabolic reserves were estimated from ten < 6 h post-emergence teneral mosquitoes, reared as described above, across the different temperature regimes and resource levels. Total soluble protein, total glycogen and carbohydrate, as well as lipid amounts were fractionated from individual insects [48]. Proteins forms the structural component, while glycogen and lipids are the major storage forms of energy. Glycogen represents the first choice for storage and use, as these are readily converted to carbohydrates, and carbohydrates are the readily available metabolic fuel for insects [59]. The amount of metabolic reserves in each individual mosquito was normalized by the respective average wing size, for each temperature and resource regime [52].

Analysis of total soluble proteins

Briefly, individual mosquitoes ($n = 10$) were placed in 2 ml microfuge tubes, macerated in 180 µl protein lysis buffer, agitated for 1 min and then centrifuged at 6700 RCF for 5 min. An aliquot of the supernatant (10 µl) was transferred to a separate microfuge tube, containing Coomassie blue dye (250 µl, Bio-Rad Laboratories AB, Solna, Sweden), vortexed for 2 min, incubated at room temperature for 17.5 ± 2.5 min and the absorbance measured at 595 nm (Multiskan FC, Thermo Fischer Scientific, Sweden). Protein content was determined using a bovine serum albumin (BSA) (Bio-Rad Laboratories AB) standard curve [49, 75].

Analysis of total carbohydrates and glycogen

To the remaining fraction of the sample (170 µl left after an aliquot of 10 µl was used for protein analysis), 20% sodium sulphate (20 µl, Merck Life Science AB, Solna, Sweden) and 1:1 methanol: chloroform (1.5 ml, both 99%, Merck Life Science AB) mixture were added and centrifuged at 6700 RCF for 5 min. The supernatant was transferred to a new microfuge tube. Total carbohydrates, in the methanol fraction, were separated from the lipid fraction, dissolved in chloroform, by the addition of 500 µl distilled water to the supernatant and then centrifuged at 6700 RCF for 5 min. Total carbohydrates in methanol fraction, and the pellet containing total glycogen were analysed using the hot anthrone reaction, and the absorbance measured at 620 nm (Multiskan FC, Thermo Fisher Scientific) [50, 75]. Total carbohydrates includes simple sugars, glucose, fructose, sucrose, oligosaccharides and all polysaccharides (which include glycogen and starch) present in the mosquitoes tissue [59]. Glycogen analysis, is specific to the amount of glycogen, a large, branched polysaccharide made entirely of glucose units and serving as a major energy storage molecule in insects [59]. By measuring both total carbohydrates and glycogen, it is possible to distinguish between readily available energy sources and long-term energy reserves in mosquito tissues [50, 59]. Amounts of total carbohydrates and glycogen were estimated from a D-glucose (99.5%, Merck Life Science AB) standard curve.

Analysis of total lipids

Lipids dissolved in the chloroform fraction were analysed in vanillin reagent (99%, Merck Life Science AB), and the absorbance measured at 520 nm (Multiskan FC, Thermo Fisher Scientific) [51, 75]. Lipid content in individual mosquitoes was quantified using olive oil standard curve [51, 75].

Feeding assay and volumetric analysis

To evaluate how temperature and resource level during larval development affects adult feeding behaviour, newly emerged females were offered either a carbohydrate- (bee honey, ICA AB, Solna, Sweden; diluted in distilled water to 60% vol/vol) or protein-rich meal (sheep blood). *Aedes aegypti* were fed during photophase (Zeitgeber time, ZT, 2–5), whereas the anophelines were fed during scotophase (ZT 13–16), analogous to their diel-activity period [53, 54]. Mosquitoes were provided either 60% honey, mixed with 1 mg ml^{-1} xylene cyanol (Merck Life Science AB), from 0.2 ml PCR strip tube caps (VWR, Stockholm, Sweden) for 3 h, or blood via membrane feeding (Hemotek Ltd) for 1 h. Blue colouration of the abdomen was used to score honey-fed from unfed insects. Ingested volumes of honey and blood from fed females were quantified by spectrophotometry, as previously described by Dawit et al. [55] and Briegel et al. [56], respectively. The volumes obtained were adjusted by the average size of females, from the respective treatments (Fig. 2). For each temperature and resource level, > 30 females were used in feeding assays, and in cases with low feeding propensity the sample size was increased so as to obtain insects for volumetric analysis.

Statistical analysis

Mixed-effects Cox regression analysis was used to estimate the effects of temperature, resource level and their interaction on the time to adult emergence. Kaplan–Meier survival curves, plotted using the survminer package, were used to visualise differences in adult survival across different experimental treatments. The effects of temperature and resource level, and their interaction on survival were determined using the mixed-effect Cox regression model [75]. Linear-mixed effects analysis of variance, using Kenward–Roger degrees of freedom approximation, was used to estimate the effects of temperature, resource level and their interaction on adult size and metabolic reserves. Pairwise comparison of emmeans was used to decipher significant interactions of the two abiotic factors across different treatments, and *P*-values were corrected by the Tukey method [57]. These analysis were conducted using the lmerTest and coxme packages [86]. Effects of temperature and resource level on feeding behaviour of newly emerged females was evaluated using the generalised linear mixed-effects model with a binomial error structure [75]. Significant interactions of the two experimental variables on the proportion of adults feeding were estimated by pairwise comparison of emmeans using Tukey method adjusted *P*-values [57]. The Aligned Rank Transform (ART) analysis of variance was used to elucidate effects of temperature, resource level and their interaction on the volume of honey and blood ingested by teneral females. The non-parametric ART analysis of variance was selected due to heteroscedasticity of the volumetric data following normality test with Shapiro–Wilcoxon analysis [65, 58]. This analysis was performed using the art function in ARTool package. Post hoc analysis of significant interactions of temperature and resource level on fitness traits was evaluated using the art.con function, and Tukey method adjusted *P*-values. In all models, replicate was treated as a random effect, and date was randomised in time to adult emergence. Temperature and resource level were modelled as fixed effects. Correlations between size and survival were determined using the non-parametric multivariate analysis of temperature, resources level, size and survival by Spearman's rank correlation. Moreover, differences in the effect of size on survival across different temperature regimes were analysed using linear regression by comparing slopes and *y*-intercepts and correcting for multiple comparisons using Tukey-adjusted *P*-values. Spearman's rank correlation and linear regression were conducted using the cor.test and lm functions, respectively [86]. All analytical tests were carried out in R (version 4.3.1, R core development team, 2023).

Results:interaction of temperature and resource level impacts life history traits of four mosquito species

Time to adult emergence

The time to adult emergence of all the four species decreased with an increase in temperature (*Ae. aegypti*: df = 2, $\chi^2 = 227.70$, $P < 0.001$; *An. stephensi*: df = 2, $\chi^2 = 296.15$, $P < 0.001$; *An. coluzzii*: df = 2, $\chi^2 = 228.39$, $P < 0.001$; *An. arabiensis*: df = 2, $\chi^2 = 183.99$, $P < 0.001$) and an increase in resource level (*Ae. aegypti*: df = 2, $\chi^2 = 698.82$, $P < 0.001$; *An. stephensi*: df = 1, $\chi^2 = 140.46$, $P < 0.001$; *An. coluzzii*: df = 1, $\chi^2 = 45.55$, $P < 0.001$; *An. arabiensis*: df = 1, $\chi^2 = 90.21$, $P < 0.001$) (Fig. 1A–D). Moreover, high larval resource level shortened the time to adult emergence of *Ae. aegypti* (Fig. 1A) and *An. coluzzii* (Fig. 1C) at high temperature (*Ae. aegypti*: df = 4, $\chi^2 = 204.89$, $P < 0.001$; *An. coluzzii*: df = 2, $\chi^2 = 21.96$, $P < 0.001$), while for *An. stephensi* (Fig. 1B) and *An. arabiensis* (Fig. 1D) this was observed across all temperature regimes (*An. stephensi*: df = 2, $\chi^2 = 20.88$, $P < 0.001$; *An. arabiensis*: df = 2, $\chi^2 = 27.31$, $P < 0.001$). At low larval resource level, *Anopheles* species larvae did not transition to pupae.

Adult size

The size of all the four species decreased with an increase in temperature (*Ae. aegypti*: $F_{2,261} = 581.14$, $P < 0.001$; *An. stephensi*: $F_{2,174} = 301.41$, $P < 0.001$; *An. coluzzii*: $F_{2,175} = 151.76$, $P < 0.001$; *An. arabiensis*: $F_{2,158} = 92.19$, $P < 0.001$) and increased with an increase in resource level (*Ae. aegypti*: $F_{2,261} = 730.55$, $P < 0.001$; *An. stephensi*: $F_{1,174} = 346.22$, $P < 0.001$; *An. coluzzii*: $F_{1,175} = 323.49$, $P < 0.001$; *An. arabiensis*: $F_{1,158} = 312.76$, $P < 0.001$) (Fig. 2A–D). Across all temperature regimes, *Ae. aegypti* (Fig. 2A) and *An. coluzzii* (Fig. 2C) females were bigger in size when maintained on a high resource level during larval development (*Ae. aegypti*:$F_{4,261} = 14.46$, $P < 0.001$; *An. coluzzii*: $F_{2,175} = 3.31$, $P = 0.039$). Temperature and resource level did not significantly interact to influence the size of *An. stephensi* ($F_{2,174} = 0.19$, $P = 0.83$; Fig. 2B) and *An. arabiensis* ($F_{2,158} = 0.27$, $P = 0.76$; Fig. 2D). When maintained on a low larval resource level, *Anopheles* species larvae did not transition to pupae, and hence no adults.

Adult survival

The survival probability of starved *Ae. aegypti* decreased with an increase in temperature (df = 2, $\chi^2 = 594.95$, $P < 0.001$) and a decrease in resource level (df = 2, $\chi^2 = 559.70$, $P < 0.001$). However, at low temperatures, *Ae. aegypti* survived longer when maintained on a high larval resource level (df = 4, $\chi^2 = 30.89$, $P < 0.001$), a pattern not observed at higher temperatures (Fig. 3A; Table 1). Temperature and resource level did not significantly interact to influence survival of the three starved anopheline species (*An. stephensi*: df = 2, $\chi^2 = 5.32$, $P = 0.070$; *An. coluzzii*: df = 2, $\chi^2 = 2.62$, $P = 0.27$; *An. arabiensis*: df = 2, $\chi^2 = 1.16$, $P = 0.56$; Fig. 3B–D; Table 1), although survival probability of *An. coluzzii* and *An. arabiensis* decreased with an increase in temperature (*An. coluzzii*; df = 2, $\chi^2 = 242.032$, $P < 0.001$; *An. arabiensis*: df = 2 $\chi^2 = 317.10$, $P < 0.001$) and a decrease in resource level (*An. coluzzii*: df = 1, $\chi^2 = 45.53$, $P < 0.001$; *An. arabiensis*: df = 1, $\chi^2 = 23.46$, $P < 0.00$) (Fig. 3C, D). While high temperature (df = 2, $\chi^2 = 242.20$, $P < 0.001$) decreased the survival probability of *An. stephensi*, resource level (df = 1, $\chi^2 = 3.42$, $P = 0.06$) did not influence the survival of this species (Fig. 3B). There was no adults of *Anopheles* species at low resource level since larvae maintained on this resource regime did not transition to pupae.

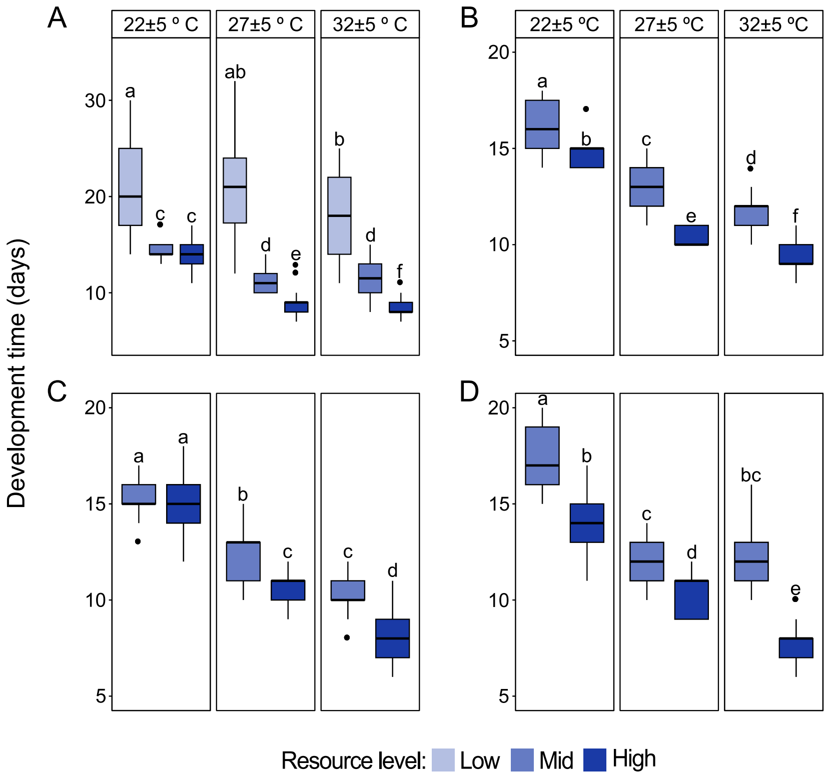


Fig. 1 Temperature and resource level interact to influence the development time of mosquitoes. Development time from egg hatching to adult emergence of female **A** *Ae. aegypti* (22 ± 5 °C: high resource *n* = 112; mid resource *n* = 105; low resource *n* = 87; 27 ± 5 °C: high resource *n* = 93; mid resource *n* = 114; low resource *n* = 85; 32 ± 5 °C: high resource *n* = 107; mid resource *n* = 94; low resource *n* = 70), **B** *An. stephensi* (22 ± 5 °C: high resource *n* = 81; mid resource *n* = 38; 27 ± 5 °C: high resource *n* = 115; mid resource *n* = 107; 32 ± 5 °C: high resource *n* = 111; mid resource *n* = 75) **C** *An. coluzzii* (22 ± 5 °C: high resource *n* = 92; mid resource *n* = 81; 27 ± 5 °C: high resource *n* = 103; mid resource *n* = 70; 32 ± 5 °C: high resource *n* = 77; mid resource *n* = 49) and **D** *An. arabiensis* (22 ± 5 °C: high resource *n* = 104; mid resource *n* = 81; 27 ± 5 °C: high resource *n* = 88; mid resource *n* = 69; 32 ± 5 °C: high resource *n* = 62; mid resource *n* = 22) in response to diurnal fluctuating temperature and resource level. Lowercase letters, from pair-wise comparisons of emmeans, indicate significant interactions of temperature and resource level on the development time ($P < 0.05$; mixed-effect Cox regression analysis). At low larval resource level *Anopheles* species did not pupate, and hence no data on development time at this resource level

Effect of size on adult survival

Survival of starved adults of all the four species significantly increased with size (*Ae. aegypti*: $\rho = 0.85$, $P < 0.0001$; *An. stephensi*: $\rho = 0.55$, $P < 0.0001$; *An. coluzzii*: $\rho = 0.73$, $P < 0.0001$; *An. arabiensis*: $\rho = 0.70$, $P < 0.0001$), although the relationship between body size and survival decreased with increasing temperature (*Ae. aegypti*: $\rho = -0.67$, $P < 0.0001$; *An. stephensi*: $\rho = -0.72$, $P = 0001$; *An. coluzzii*: $\rho = -0.70$, $P < 0.0001$; *An. arabiensis*: $\rho = -0.75$, $P < 0.0001$;Additional file 1: Supplementary Fig. S3A–D). The effect of size on survival of starved *Ae. aegypti* ($F_{2,856} = 72.41$, $P < 0.0001$) was higher than that of the *Anopheles* species (*An. stephensi*: $F = _{2,524} = 9.1$, $P < 0.0001$; *An. arabiensis*: $F_{2,433} = 9.28$, $P < 0.0001$; *An. coluzzii*: $F_{2,469} = 10.91$, $P < 0.0001$), but the effect decreased with increasing temperature (Additional file 1: Supplementary Fig. S3A–D; Additional file 2: Supplementary Table S1). The effect of size on survival of starved *Ae. aegypti* and *An. arabiensis* significantly decreased with an increase in temperature up to 27 °C, whereas at 32 °C this effect was low. Conversely, for *An. stephensi* and *An. coluzzii* the size-dependent survival decreased at the highest

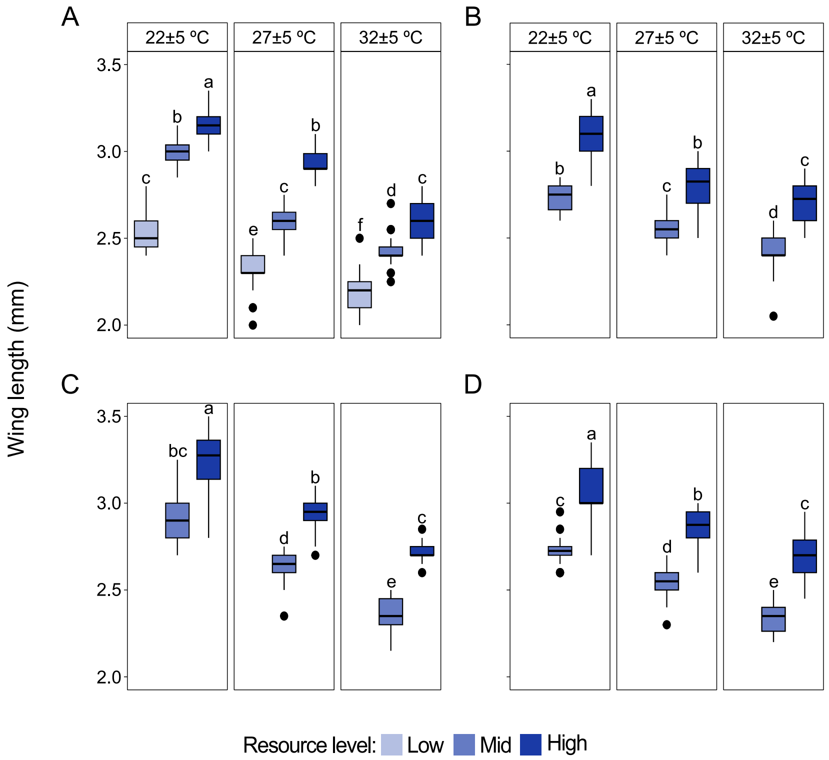


Fig. 2 Temperature and resource level interact to influence the adult size of mosquitoes. The wing length of female **A** *Aedes aegypti*, **B** *Anopheles stephensi*, **C** *Anopheles coluzzii* and **D** *Anopheles arabiensis* was used as a proxy for adult size. For all species, $n = 30$ in each treatment regime. Lowercase letters, from pair-wise comparisons of emmeans, indicate significant interactions of temperature and resource level on the size for each species. Bars followed by different letters within the same box (between resource levels and same temperature) and bars across boxes (between temperature and same resource) are statistically different when represented by different letters ($P < 0.05$; Mixed-effect ANOVA). At low larval resource level *Anopheles* species did not pupate, and hence no data on size at this resource level

temperature, 32 °C (Additional file 2: Supplementary Table S1). The effect of size on survival of starved adults of all species significantly increased with an increase in resource level (*Ae. aegypti*: $\rho = 0.53$, $P < 0.0001$; *An. stephensi*: $\rho = 0.13$, $P = 0036$; *An. coluzzii*: $\rho = 0.32$, $P < 0.0001$; *An. arabiensis*: $\rho = 0.21$, $P < 0.0001$), in which larger females emerging from high larval resource regime lived longer compared with smaller females from low-resource level.

Metabolic reserves

Metabolic reserves carried over from the larval to adult stage of the four species were determined by the interaction of temperature and resource level during aquatic development (Fig. 4A–P). The total soluble protein (Fig. 4A) in teneral *Ae. aegypti* increased with an increase in temperature when females were maintained on mid resource level ($F_{4,54} = 18.06$, $P < 0.001$). However, at high temperature this macronutrient decreased in insects maintained on a high resource level (Fig. 4A). Total carbohydrates content (Fig. 4B) was high in females reared under low temperature and maintained on a mid resource level, while a decrease in this reserve was observed at intermediate temperature in insects maintained on a similar resource regime ($F_{4,54} = 16.71$, $P < 0.001$). Moreover, at high temperature, the glycogen content (Fig. 4C) in teneral *Ae. aegypti* decreased in

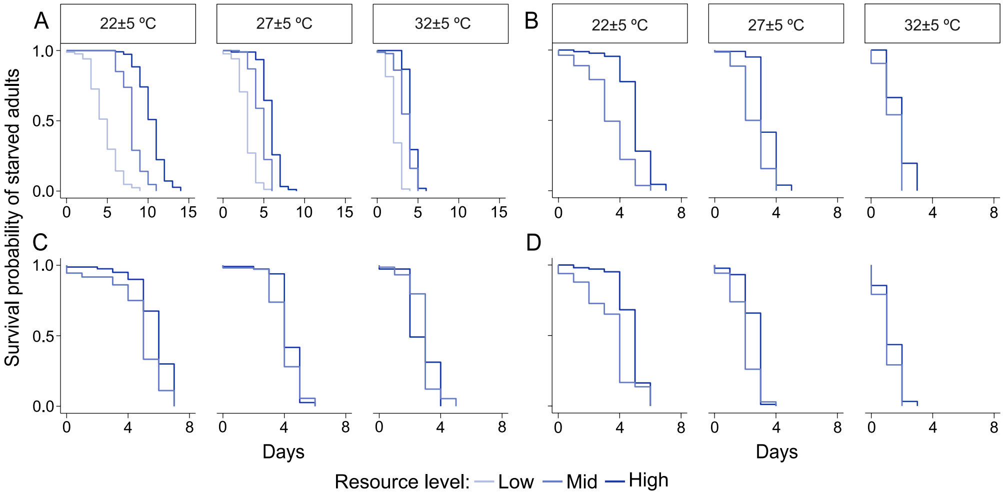


Fig. 3 Temperature and resource level interact to influence the adult survival of mosquitoes. Adult survival of female **A** *Ae. aegypti*, (22 ± 5 °C: high resource *n* = 112; mid resource *n* = 105; low resource *n* = 87; 27 ± 5 °C: high resource *n* = 93; mid resource *n* = 114; low resource *n* = 85; 32 ± 5 °C: high resource *n* = 107; mid resource *n* = 94; low resource *n* = 70), **B** *An. stephensi* (22 ± 5 °C: high resource *n* = 81; mid resource *n* = 38; 27 ± 5 °C: high resource *n* = 115; mid resource *n* = 107; 32 ± 5 °C: high resource *n* = 111; mid resource *n* = 75), **C** *An. coluzzii* (22 ± 5 °C: high resource *n* = 92; mid resource *n* = 81; 27 ± 5 °C: high resource *n* = 103; mid resource *n* = 70; 32 ± 5 °C: high resource *n* = 77; mid resource *n* = 49) and **D** *An. arabiensis* (22 ± 5 °C: high resource *n* = 104; mid resource *n* = 81; 27 ± 5 °C: high resource *n* = 88; mid resource *n* = 69; 32 ± 5 °C: high resource *n* = 62; mid resource *n* = 22) in response to diurnal fluctuating temperature and resource level. Significant interactions of temperature and resource level on adult survival are shown in Table 1. At low larval resource level *Anopheles* species did not pupate, and hence no data on survival at this resource level

Table 1 Mixed-effect Cox regression analysis on the interactive effect of temperature and resource level on adult mosquito survival

Species	Temperature (± 5 °C)	Resource level (mg larva^{-1} day^{-1})		
		0.1	0.32	1
Aedes aegypti	22	cd	b	a
	27	f	d	c
	32	g	e	e
Anopheles stephensi	22	–	a	a
	27	–	b	b
	32	–	c	c
		(mg larva^{-1} day^{-1})		
		0.075	0.24	0.75
Anopheles arabiensis	22	–	b	a
	27	–	c	c
	32	–	e	e
Anopheles coluzzii	22	–	b	a
	27	–	c	b
	32	–	e	d

Different alphabetical letters indicate significant interaction between temperature and resource level for each species ($P < 0.05$)

females reared on a high resource level, while this macronutrient increased in females maintained on mid larval resource level ($F_{4,54} = 14.50$, $P < 0.001$). Total soluble lipid content (Fig. 4D) increased when teneral females were reared under low temperature and maintained on a high- compared with low-larval resource level ($F_{4,54} = 11.14$, $P < 0.001$).

As temperature increased, the total soluble protein (Fig. 4E), glycogen (Fig. 4G) and lipids (Fig. 4H) increased in teneral *An. stephensi* reared on mid larval resource level (proteins: $F_{2,26} = 15.03$, $P < 0.001$; glycogen: $F_{2,27} = 22.04$, $P < 0.001$; lipids: $F_{2,27} = 49.40$, $P < 0.001$), while at low temperature the lipid content decreased in females maintained on a similar resource level (Fig. 4H). Across all temperature regimes, the total carbohydrates (Fig. 4F) increased in teneral *An. stephensi* maintained on mid resource level during larval development ($F_{2,54} = 9.77$, $P < 0.001$). Conversely, temperature and resource level did not significantly interact to determine the protein (Fig. 4I) and glycogen (Fig. 4K) content (protein: $F_{2,27} = 0.62$, $P = 0.54$; glycogen:$F_{2,27} = 2.92$, $P = 0.071$) in *An. coluzzii*, although across all temperatures, and at high temperature, protein and glycogen, respectively, increased in teneral females maintained on mid- compared with high-resource level during larval development. However, as temperature increased, total carbohydrates (Fig. 4J) increased when teneral *An. coluzzii* were reared on high larval resource level, whereas at low temperature this macronutrient decreased in females maintained on a similar resource level ($F_{2,27} = 66.06$, $P < 0.001$). Total soluble lipid content (Fig. 4L) increased in teneral females reared under high- compared with intermediate-temperature when larvae were maintained on mid larval resource level ($F_{2,27} = 6.27$, $P < 0.01$). As temperature decreased, total soluble protein content (Fig. 4M) in female *An arabiensis* increased when reared on mid larval resource level ($F_{2,26} = 21.78$, $P < 0.001$), whereas total carbohydrates (Fig. 4N) and glycogen (Fig. 4O) increased in teneral females maintained on a high resource level during larval development (carbohydrates: $F_{2,26} = 13.86$, $P < 0.001$; glycogen: $F_{2,26} = 14.58$, $P < 0.001$). While at intermediate temperature the total soluble lipids (Fig. 4P) decreased in teneral *An. arabiensis* maintained on mid larval resource level, generally, as temperature increased this macronutrient increased in females reared on mid resource level ($F_{2,26} = 4.086$, $P = 0.028$).

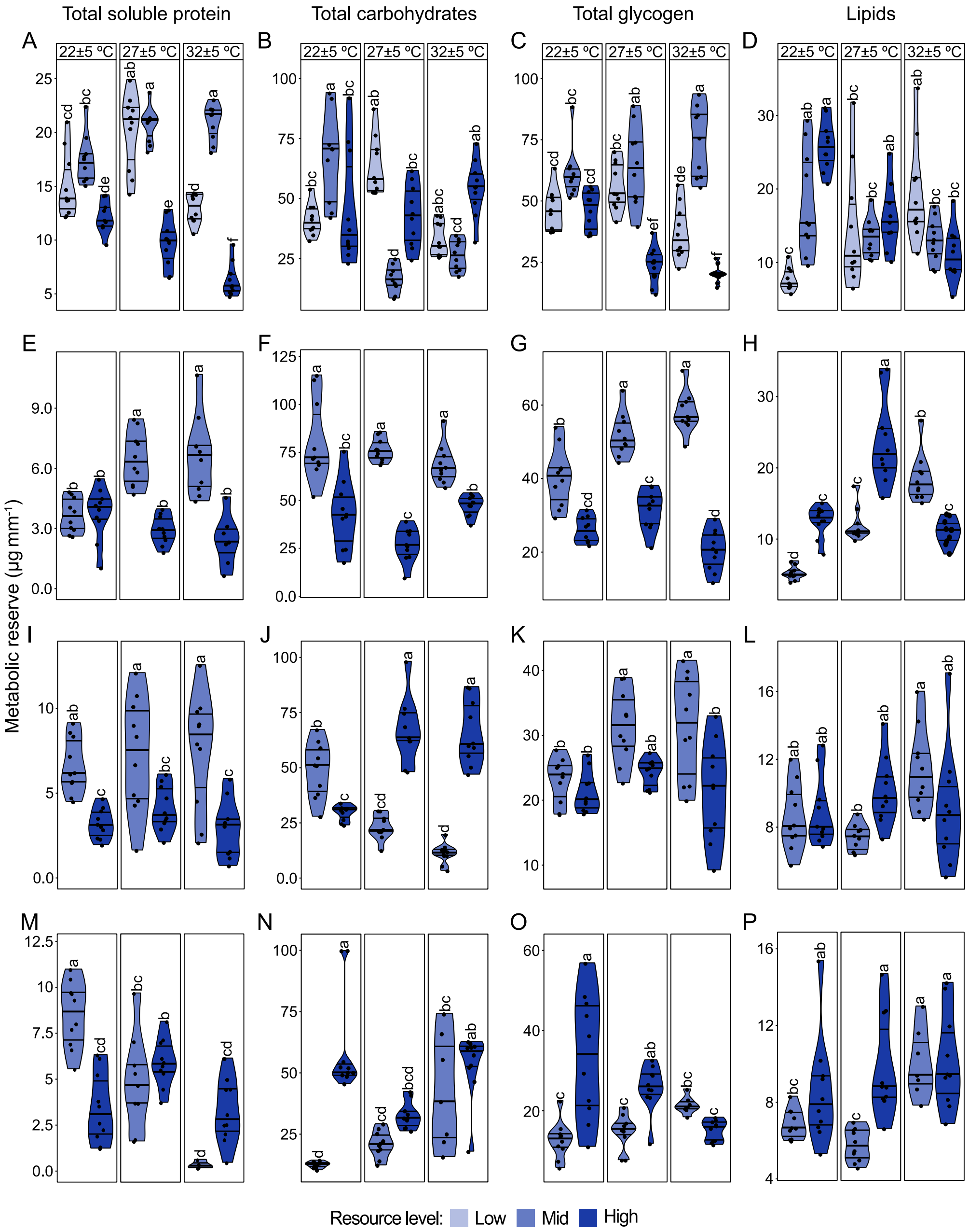
Propensity to feed on honey or blood

The propensity of teneral females to replenish metabolic reserves with a carbohydrate meal (honey) was differentially influenced by temperature, with larval resource level negatively affecting the temperature effect in a species-dependent manner (Fig. 5A–H). While temperature and resource level during larval development did not significantly interact to influence proportion of females feeding (*Ae. aegypti*; df = 4, $\chi^2 = 4.68$, $P = 0.32$; *An. coluzzii*; df = 2, $\chi^2 = 1.59$, $P = 0.45$ and *An. arabiensis*; df = 2, $\chi^2 = 3.20$, $P = 0.20$; Fig. 5A, C, D), a higher proportion of *Ae. aegypti* and *An. coluzzii* fed as temperature increased (*Ae. aegypti*: df = 2, $\chi^2 = 41.92$, $P < 0.001$; *An. coluzzii*: df = 2, $\chi^2 = 13.01$, $P < 0.01$) an effect accentuated by restricted larval resource level (*Ae. aegypti*: df = 2, $\chi^2 = 12.10$, $P < 0.01$; *An. coluzzii*: df = 1, $\chi^2 = 15.33$, $P < 0.001$) (Fig. 5A, C). In contrast, temperature (df = 2, $\chi^2 = 21.56$, $P < 0.001$), but not resource level (df = 1, $\chi^2 = 0.18$, $P = 0.20$) enhanced the proclivity of *An. arabiensis* to feed on honey (Fig. 5D). Honey feeding by *An. stephensi* increased at high temperature when food resource level was restricted (df = 2, $\chi^2 = 12.60$, $P < 0.01$; Fig. 5B). Resource level and temperature significantly influenced the level of feeding by *Ae. aegypti* (Fig. 5E) and *An. coluzzii* (Fig. 5G), respectively (Additional file 2: Supplementary Table S2), in which *Ae. aegypti* females from high resource level fed more, whereas the level of feeding by *An. coluzzii* decreased at high temperature and resource level. The volume of honey ingested by *An. stephensi* (Fig. 5F) and *An. arabiensis* (Fig. 5H), on the other hand, was not affected by the two environmental factors (Additional file 2: Supplementary Table S2).

Temperature and larval resource level significantly interacted to influence the proclivity of teneral *An. coluzzii* (df = 2, $\chi^2 = 18.88$, $P < 0.001$; Fig. 5K) but not *Ae. aegypti* (df = 4, $\chi^2 = 3.21$, $P = 0.52$; Fig. 5I) and *An. stephensi* (df = 2, $\chi^2 = 0.076$, $P = 0.96$; Fig. 5J) to feed on a proteinaceous meal (blood), although feeding by the

(See figure on next page.)

Fig. 4 Temperature and resource level interact to influence the teneral metabolic reserves of mosquitoes. Metabolic macronutrients adjusted by mean wing size, in teneral female (**A**-**D**) *Ae. aegypti*, (**E**-**H**) *An. stephensi*, (**I**-**L**) *An. coluzzii* and (**M**-**P**) *An. arabiensis*. For all species, $n = 10$ in each treatment regime. Lowercase letters, from pair-wise comparisons of emmeans, indicate significant interactions of temperature and resource level on the macronutrient for each species. Bars followed by different letters within the same box (between resource levels and same temperature) and bars across boxes (between temperature and same resource) are statistically different when represented by different letters ($P < 0.05$; mixed-effect ANOVA). At low larval resource level *Anopheles* species did not pupate, and hence no data on macronutrients at this resource level



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three species increased significantly at intermediate temperature compared with low and high temperature regimes, an effect accentuated by higher larval resource levels.

The effect of temperature and resource regimes during larval development influenced the volume of blood ingested as reflected by the proportion of *Ae. aegypti*, *An. stephensi* and *An. coluzzii* that fed. Temperature and larval resource level did not interact to influence the amount engorged by *An. stephensi* ($F_{2,104}=0.99$, $P=0.37$), *Ae. aegypti* ($F_{4,166}=0.27$, $P=0.90$) and *An. coluzzii* ($F_{2,58}=0.59$, $P=0.56$). In female *Ae. aegypti* (Fig. 5M) and *An. stephensi* (Fig. 5N), the volume was positively correlated with temperature and resource level while a similar trend of resource, but not temperature, was observed for *An. coluzzii* (Fig. 5O). The propensity of *An. arabiensis* (Fig. 5L) to feed on blood appears inversely proportionate to temperature, irrespective of the resource level during larval development, although no significant effect of the interaction of the two factors was observed ($F_{2,12}=45.40$, $P=0.88$). Moreover, while temperature and resource regimes during larval development did not significantly interact to influence the level of feeding ($F_{2,154}=1.29$, $P=0.28$), the amount ingested decreased with an increase in temperature, an effect accentuated by larval resource restriction (Fig. 5P).

Discussion

Fluctuating diurnal temperature modulated mosquito life history traits, an effect accentuated by food availability during larval development, supporting previous studies performed under constant temperature [19, 30]. However, the response of the aquatic stages to the two environmental factors is taxon-dependent, resulting in differential carry-over effects in the adults, reflected in differential metabolic reserves and propensity to feed, as demonstrated in the current study. This may have significant and differential consequences for population dynamics and thus the vectorial capacity of mosquitoes.

Aquatic stages of mosquitoes reared at high temperatures, have a shorter developmental time likely owing to accelerated physiological processes, such as metabolic rates leading to a faster progression through the aquatic stages [4, 23, 24, 60–62]. The availability of food accentuates the observed temperature effects on time to adult emergence [19, 30, 62, 66]. Resource restriction during larval development significantly increased the time to adult emergence, while abolishing adult *Anopheles*

(See figure on next page.)

Fig. 5 Temperature and resource level interact to influence the feeding propensity of teneral mosquitoes. Proportion of teneral female feeding on either a carbohydrate (honey) **A** *Ae. aegypti* (22 ± 5 °C: high resource *n* = 36; mid resource *n* = 35; low resource *n* = 46; 27 ± 5 °C: high resource *n* = 61; mid resource *n* = 40; low resource *n* = 36; 32 ± 5 °C: high resource *n* = 72; mid resource *n* = 39; low resource *n* = 37), **B** *An. stephensi* (22 ± 5 °C: high resource *n* = 54; mid resource *n* = 46; 27 ± 5 °C: high resource *n* = 55; mid resource *n* = 46; 32 ± 5 °C: high resource *n* = 52; mid resource *n* = 75), **C** *An. coluzzii* (22 ± 5 °C: high resource *n* = 32; mid resource *n* = 59; 27 ± 5 °C: high resource *n* = 76; mid resource *n* = 53; 32 ± 5 °C: high resource *n* = 49; mid resource *n* = 35) and **D** *An. arabiensis* (22 ± 5 °C: high resource *n* = 32; mid resource *n* = 54; 27 ± 5 °C: high resource *n* = 52; mid resource *n* = 63; 32 ± 5 °C: high resource *n* = 51; mid resource *n* = 40) or proteinaceous (blood) meal **I** *Ae. aegypti* (22 ± 5 °C: high resource *n* = 37; mid resource *n* = 32; low resource *n* = 30; 27 ± 5 °C: high resource *n* = 49; mid resource *n* = 89; low resource *n* = 37; 32 ± 5 °C: high resource *n* = 67; mid resource *n* = 30; low resource *n* = 36), **J** *An. stephensi* (22 ± 5 °C: high resource *n* = 46; mid resource *n* = 44; 27 ± 5 °C: high resource *n* = 30; mid resource *n* = 60; 32 ± 5 °C: high resource *n* = 43; mid resource *n* = 30), **K** *An. coluzzii* (22 ± 5 °C: high resource *n* = 42; mid resource *n* = 32; 27 ± 5 °C: high resource *n* = 57; mid resource *n* = 30; 32 ± 5 °C: high resource *n* = 53; mid resource *n* = 25) and **L** *An. arabiensis* (22 ± 5 °C: high resource *n* = 30; mid resource *n* = 39; 27 ± 5 °C: high resource *n* = 36; mid resource *n* = 40; 32 ± 5 °C: high resource *n* = 105; mid resource *n* = 16). Lowercase letters, from pair-wise comparisons of emmeans, indicate significant interactions between temperature and resource level on honey and blood feeding. Bars followed by different letters within the same box (between resource levels and same temperature) and bars across boxes (between temperature and same resource) are statistically different when represented by different letters ($P < 0.05$; generalised mixed-effect binomial analysis). Volume, adjusted by mean wing size, of honey ingested by teneral **E** *Ae. aegypti* (22 ± 5 °C: high resource *n* = 2; mid resource *n* = 3; low resource *n* = 5; 27 ± 5 °C: high resource *n* = 13; mid resource *n* = 23; low resource *n* = 27; 32 ± 5 °C: high resource *n* = 30; mid resource *n* = 27; low resource *n* = 21), **F** *An. stephensi* (22 ± 5 °C: high resource *n* = 5; mid resource *n* = 2; 27 ± 5 °C: high resource *n* = 21; mid resource *n* = 18; 32 ± 5 °C: high resource *n* = 24; mid resource *n* = 22), **G** *An. coluzzii* (22 ± 5 °C: high resource *n* = 3; mid resource *n* = 17; 27 ± 5 °C: high resource *n* = 41; mid resource *n* = 46; 32 ± 5 °C: high resource *n* = 22; mid resource *n* = 30) and **H** *An. arabiensis* (22 ± 5 °C: high resource *n* = 4; mid resource *n* = 12; 27 ± 5 °C: high resource *n* = 18; mid resource *n* = 17; *n* = ; 32 ± 5 °C: high resource *n* = 32; mid resource *n* = 20) or blood ingested by teneral female **M** *Ae. aegypti* (22 ± 5 °C: high resource *n* = 22; mid resource *n* = 16; low resource *n* = 3; 27 ± 5 °C: high resource *n* = 33; mid resource *n* = 45; low resource *n* = 17; 32 ± 5 °C: high resource *n* = 23; mid resource *n* = 13; low resource *n* = 3), **N** *An. stephensi* (22 ± 5 °C: high resource *n* = 19; mid resource *n* = 5; 27 ± 5 °C: high resource *n* = 24; mid resource *n* = 25; 32 ± 5 °C: high resource *n* = 31; mid resource *n* = 6), **O** *An. coluzzii* (22 ± 5 °C: high resource *n* = 4; mid resource *n* = 14; 27 ± 5 °C: high resource *n* = 36; mid resource *n* = 7; 32 ± 5 °C: high resource *n* = 2; mid resource *n* = 1) and **P** *An. arabiensis* (22 ± 5 °C: high resource *n* = 22; mid resource *n* = 32; 27 ± 5 °C: high resource *n* = 35; mid resource *n* = 28; *n* = ; 32 ± 5 °C: high resource *n* = 40; mid resource *n* = 4). Lowercase letters, from pair-wise comparisons of emmeans, indicate significant interactions between temperature and resource level on the volume ingested. Bars followed by different letters within the same box (between resource levels and same temperature) and bars across boxes (between temperature and same resource) are statistically different when represented by different letters ($P < 0.05$; Aligned Rank Transform analysis of variance). At low larval resource level *Anopheles* species did not pupate, and hence no data on feeding propensity at this resource level

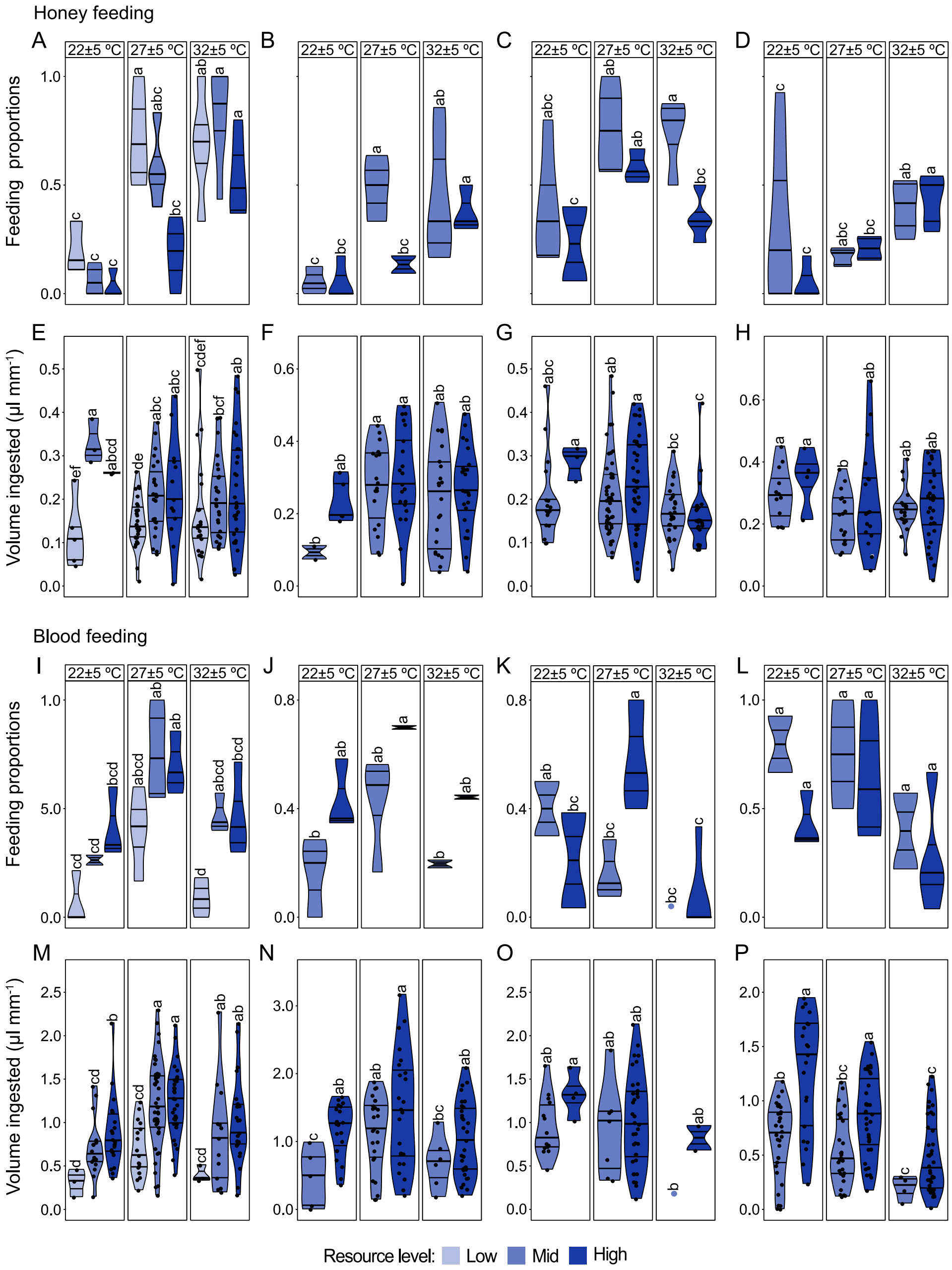

Fig. 5 (See legend on previous page.)

emergence. This is likely owing to the requirement for the larvae to extend their feeding period to accumulate sufficient reserves to complete pupation [67]. The reason that *Ae. aegypti* is more tolerant to food restriction compared with the anopheline species is likely due to a better capacity to amass metabolic reserves under elevated temperature conditions, as well as differences in nutritional requirements between genera [36, 37, 52, 68, 85], which translates into differential carry-over effects in the adults.

The carry-over effects of temperature stress during the aquatic stages were evidenced by reduced adult size and survival [24, 29, 61, 62], in line with the "size and fitness rule" [4, 69], which states that bigger is better. This effect was accentuated by decreasing food resource quantity during the larval stages [19, 30]. When survival under temperature stress is independent of food stress during the non-reproductive state, *i.e.*, the larval stage in the case of this study, the carry-over effects to the reproductive state, affecting fitness, may produce a stabilising effect on populations over time [71]. These mosquitoes are likely to survive as adults largely independently of food restriction during the aquatic stages. Furthermore, behavioural adaptation may allow mosquitoes to escape high temperatures. For instance, *An. coluzzii* aestivate during hot and dry months [72] and *Ae. aegypti* seek refuges in cool human habitats [2, 73, 74].

The teneral reserves, potentially regulating the survival of adults, were differentially affected by prior temperature stress and accentuated by resource restriction during the aquatic stage. Protein content generally reflects the structural biomass of mosquitoes, *i.e.*, size; however, protein can also be used as a metabolic fuel during starvation [36, 37] and abiotic stress [75]. As the protein content was normalised for the size of the female in the current study, the effects of the environmental stressors, particularly food abundance, were relatively minor within species. The genus-dependent differences in the amount of protein carried-over into the adult stage relate to disparities between *Ae. aegypti* and *Anopheles* species accumulation and utilisation of protein from the teneral reserves. *Aedes aegypti* and *Anopheles* species accumulate protein during larval development, rendering them either 'obese' [68] or 'undernourished' [36], respectively, which likely restricts the eclosion of *Anopheles* at low-level diet conditions, as also reflected in the current study. In response to stress, particularly food abundance, teneral protein content is differentially affected, with *Ae. aegypti* being able to mobilise three times less of their protein reserves than *Anopheles* [36, 37]. This correlates with a shorter life span for *Ae. aegypti* under stress.

While mosquitoes utilise protein for energy under stress, the primary metabolites relied on for energy are lipids and glycogen [36, 37]. A high content of the two long-term reserves may increase adult longevity and starvation resilience [59, 76, 77]. Survival directly correlates with adult size, with the correlation weakening with increasing temperature across all species [4, 19, 78]. At the highest temperatures, the *Anopheles* species continue to increase in size, if provided ample food as larvae, but no longer increase in adult longevity [4], indicating that the similar levels of teneral reserves across species are independent of stress conditions. This is reminiscent of the 'energy limited tolerance to stress' concept, in which more energy is needed for the basal maintenance of large insects to cope with temperature stress' [79] and the need to allocate energy reserves for reproduction [4, 19, 78]. To alleviate any shortage in teneral reserves, adult females engage in feeding on different nutritional sources.

Females fed differentially on carbohydrate- and protein-rich meals, emphasising species-dependent strategies in replenishing metabolic reserves, in response to temperature- and resource-induced stress. In contrast to other insects studied, which regulate the carbon:nitrogen balance through quantities ingested [80, 81], mosquitoes separate the carbon-rich meal from the nitrogen-rich meal spatially and temporally depending on their physiological status [70], thus expanding the regulation from amount ingested to the proportion of individuals actively engaged in feeding on either resource. In response to an increase in temperature and resource restriction, females of all species had a high propensity to feed on honey demonstrating a reliance on sugar sources in response to stress. The propensity of teneral females to feed on blood was independent of temperature and resource level during larval development, whereas the volume of blood ingested was dependent on species as well as temperature and resource level during larval development. The volume of blood engorged by the teneral female is inversely proportional to the carbohydrate reserves, and regulated by temperature and resource level during larval development, indicating that while mosquitoes separate their carbohydrate- and protein-rich meals, the females appear to follow the canonical balancing model for carbon and nitrogen [70]. Taken together, female mosquitoes appear to engage in species-dependent feeding to replenish and balance metabolic reserves in response to abiotic stress.

Conclusions

This study underscores the effects of temperature stress and diet limitation on species-dependent life history traits of aquatic and terrestrial stages of mosquitoes.

A shorter development time at high temperature, independent of larval food resources, may increase population growth rate and stability, which may increase disease transmission [39, 69]. Disease transmission dynamics is, furthermore, likely to be influenced by species-dependent strategies and capacities to store metabolic reserves in response to biotic and abiotic stress [29, 36], which has ramifications for teneral adult feeding behaviour. Abiotic and biotic stress may both increase or decrease the avidity of females to blood feed, which may affect the human biting rate and hence, disease transmission [12, 35, 39, 82–84]. The comparative assessment of how different mosquito species respond to abiotic stress provides an insight to how vector fitness is affected, and needs to be incorporated into mechanistic prediction models to provide a holistic understanding of mosquito-borne disease transmission dynamics in response to climate change. We acknowledge that species used in this study originated from long standing laboratory colonies; therefore, their response to abiotic stress might differ from that of field populations. In addition, resource levels used in this study were not varied per larval instar, and hence larvae overfeeding at high resource level [85] may confound the observed effects of abiotic stress on different life history parameters, and this requires further investigation.

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s13071-026-07313-4.

Additional file 1: Supplementary Figure S1. A schematic representation of experimental set up on the effect of temperature and larval resource level on (A) immature and adult life history traits, (B) teneral metabolic reserves and (C) feeding propensity of teneral adults. Supplementary Figure S2. Schematic representation of diurnal temperature fluctuation in the experimental chambers during the experimental photoperiod. White and shaded areas represent photophase and scotophase, respectively. ZT: Zeitgeber time. Temperature increases gradually to a maximum during photophase and decreases to a minimum during scotophase. The low temperature fluctuates at a range of 17-27 °C around a mean of 22 °C, intermediate temperature at 22-32 °C around a mean of 27 °C and high temperature at 27-37 °C around a mean of 32 °C. Supplementary Figure S3. Correlation between survival and size of (A) Ae. aegypti, (B) An. stephensi, (C) An. coluzzii and (D) An. arabiensis in response to diurnal fluctuating temperature and larval resource level. R2 represents the Pearson's correlation coefficient. Error bars are constructed using 95% confidence interval (CI) to indicate variation of the mean survival duration. The grey shading indicates the 95% CI margin.

Additional file 2: Supplementary Table S1. Pairwise linear regression analyses following the findings of significant overall models of the effect of size on survival of each mosquito species at different temperature regimes (P<0.05). Supplementary Table S2. Aligned Rank Transform analysis of variance on temperature, resource level, and their interaction on the volume of honey (carbohydrate meal) ingested by each teneral mosquito species (P<0.05).

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Author contributions
JWJ designed the study; JWJ collected the data, analysed the data, drafted and revised the manuscript. MG revised the manuscript and LG-B was involved in supervision, with all authors giving final approval for publication.

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Data availability
All data can be found in the Github repository: https://github.com/JuliahJacob/Effect-of-abiotic-stress-on-the-life-history-and-behaviour-of-diseasetransmitting-mosquitoes.

Declarations

Ethics approval and consent to participate
Not applicable.

Consent for publication
Not applicable.

Competing interests
The authors declare that they have no competing interests.

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Temperature determines fitness traits of mosquitoes, and food resource abundance in aquatic habitats. This thesis explores how these two environmental factors interact to determine fitness and feeding of homo- and hetero-specific mosquito species using *Aedes aegypti, Anopheles stephensi, Anopheles coluzzii* and Anopheles arabiensis. Findings from this study underscore the effects of biotic and abiotic stress on mosquito fitness traits and behaviour that are key to population growth rate, community structure and vectorial capacity.

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