THE INFLUENCE OF TEMPERATURE AND AGE-DEPENDENT SURVIVAL ON *ANOPHELES GAMBIAE S.S.* LIFE-HISTORY AND POPULATION DYNAMICS

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DECLARATION OF ORIGINALITY

I declare that the work presented here, as part of this thesis, is my own work, carried out and completed under the supervision of Dr. Paul Parham, Prof. María-Gloria Basáñez, and Prof. Jacob Koella. All researchers and collaborators who shared their data, and advised or commented upon the analyses or the manuscript have been acknowledged. All other research and studies mentioned in the thesis have been appropriately referenced.

Céline Christiansen-Jucht January 2016

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ABSTRACT

Malaria transmission depends on the life-history parameters, and population dynamics, of its vectors, and particularly on the survival of adult *Anopheles* mosquitoes, the main vector of malaria in Africa. These dynamics are sensitive to climatic and environmental factors, and temperature is a particularly important driver, though the exact extent of this sensitivity is not well established. Some data currently exist on the influence of constant and fluctuating environmental temperature on *Anopheles gambiae* s.s. survival but very few exist examining other life-history traits, and none exist on the influence of larval temperature on adult life-history.

This thesis conducts a detailed investigation into the effect of temperature on life-history parameters of *Anopeheles gambiae* s.s., and uses experimental results to inform the structure and parameterisation of a suite of mathematical models of mosquito population dynamics.

Increasing environmental temperature during the larval stages is shown to decrease larval survival, and significantly increase adult mortality. Higher environmental temperature during the adult stages also significantly lowers adult survival. The data also highlight that mosquitoes senesce, and that age-dependent survival models fit experimental data best. Environmental temperature during the larval and adult stages is also shown to have an effect on a number of other life-history parameters.

The set of novel mathematical models of climate-driven mosquito population dynamics developed here are motivated by the experimental data suggesting that in *An. gambiae*, mortality is temperature and age dependent, and show that age-dependent models consistently fit the data better than the reference model.

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ABBREVIATIONS

An. gambiae	*Anopheles gambiae*
AIC	Akaike information criterion
AICc	corrected Akaike information criterion
BIC	Bayesian information criterion
CI	confidence intervals
DD	degree-days
EV	erythrocytic vaccines
IPCC	Intergovernmental Panel on Climate Change
IRS	indoor residual spraying
ITN	insecticide-treated nets
LLIN	long-lasting insecticidal nets
LSE	least squares estimation
LSM	larval source management
MLE	maximum likelihood estimation
MTD	minimum temperature for development
ODE	ordinary differential equation
PE	protective efficacy
PEV	pre-erythrocytic vaccines
RH	relative humidity
s.s.	sensu stricto
sic	sea-ice coverage
SIT	sterile insect technique
sst	sea-surface temperature
TBV	transmission-blocking vaccines
VBD	vector-borne disease
WHO	World Health Organization
wrt	with respect to

CHAPTER 1

INTRODUCTION

1. Background

Current evidence shows an increase in global surface and atmospheric temperatures that points to a long-term change in the earth's climate (Internation Panel on Climate Change 2013; Houghton et al. 2001). This shift is expected to influence environmental factors, such as temperature, rainfall, and desiccation, as well as social and economic factors such as human and animal population movement (IPCC 2013), which will have important repercussions for human health (Patz et al. 2003; McMichael et al. 2003). Waterborne diseases and vector-borne diseases (VBDs) are particularly sensitive to changes in environmental variables (IPCC 2007; Caminade et al. 2010), as climatic factors, and fluctuations in climatic factors, significantly affect the distribution and the population dynamics of insect vectors of VBDs by influencing their survival, reproduction and development, ecology, and susceptibility to infection (Haines et al. 2006; Mills et al. 2010; Sutherst 2004; Beugnet & Chalvet-Monfray 2013). For example, malaria, a VBD caused by *Plasmodium* parasites and transmitted by mosquitoes, is expected to be sensitive to climate shifts due to the cold-blooded mosquito's sensitivity to temperature and humidity, and to the malaria parasite's temperature-dependent development within the mosquito (Caminade et al. 2014; Piontek et al. 2014; Morse et al. 2009).

One of the main research aims now is to determine how current climatic factors influence the distribution, survival, and reproduction of the mosquito vectors of malaria, and to what extent this affects the spread of malaria, both now and under changing climate scenarios. This requires both a thorough understanding of the life history and the population dynamics of malaria vectors, and powerful theoretical tools such as mathematical modelling to provide precise predictions and simulations on varying scales. This thesis presents the results of experimental investigation into the effect of temperature on the primary vector of malaria in Africa, *Anopheles gambiae* s.s., and the consequences of incorporating these experimental results into models of vector population dynamics, in the hope that this research may help to highlight target areas for future experimental research, and be utilised to improve current models and modelling assumptions on the effect of climate on vector abundance.

2. Literature review

2.1. Malaria

Malaria is one of the most widespread and severe infectious diseases in the world, and is responsible for an estimated one million deaths every year, mostly children under 5 years old, and 20% of all deaths in Sub-Saharan Africa (UNICEF 2005; WHO 2010). Malaria also has a population-wide impact in addition to the high childhood mortality rate. Infected individuals living in areas with high levels of malaria transmission may fall sick regularly and therefore unable to work and earn a steady income, resulting in poverty for the individual and their family, as well as economic loss on a wider scale. In malaria-endemic countries, it is estimated that the burden of malaria accounts for a decrease of up to 1.3% of the gross domestic product (WHO 2010). For this reason much emphasis is placed on combating malaria as part of the Millennium Development Goals (UNDP 2011) through methods including better and more widespread treatment of the disease, interrupting transmission, and controlling the vector population (WHO 2014).

2.2. Natural history of malaria

Malaria is a vector-borne disease, caused by a parasite of the genus *Plasmodium* (Apicomplexa: Plasmodidae). Of the 100 species of *Plasmodium* infecting various animal species, only five are currently known to infect humans: *P. falciparum*, *P. vivax*, *P. ovale*, *P. malariae*, and *P. knowlesi* (Tuteja 2007; Kantele & Jokiranta 2011). The parasites have extremely complex, albeit similar, lifecycles (Fig. 1.1), the main difference being that while *P. vivax* and *P. ovale* can remain dormant in the human liver for weeks up to many years, and *P. malariae* can remain in the bloodstream for up to decades, *P. falciparum* is unable to remain dormant in the host (Tuteja 2007).

An infected female *Anopheles* mosquito bites a human and in the process injects *Plasmodium* sporozoites into the bloodstream. These migrate directly to the liver, where they start to divide and multiply, and develop into schizonts within the hepatocytes. Eventually the schizonts release merozoites into the bloodstream, initiating the erythrocytic part of the lifecycle (Pouniotis et al. 2004). Most merozoites will then invade individual red blood cells, and continue their maturation process inside the erythrocyte, where the merozoites undergo asexual division. After further maturation and transformation into a trophozoite, the parasites undergo divisions of the nucleus to become schizonts. Every schizont contains a further 20 merozoites. These are released upon lysis of the erythrocyte, and they are then free to infect

new red blood cells. This cyclical process of lysis and release of merozoites together with malarial pigment, called the erythrocytic cycle, causes the physical symptoms of (tertian or quartan) fever in the human host (Jotte & Scott 1993; Schwartz 2003; Tuteja 2007).

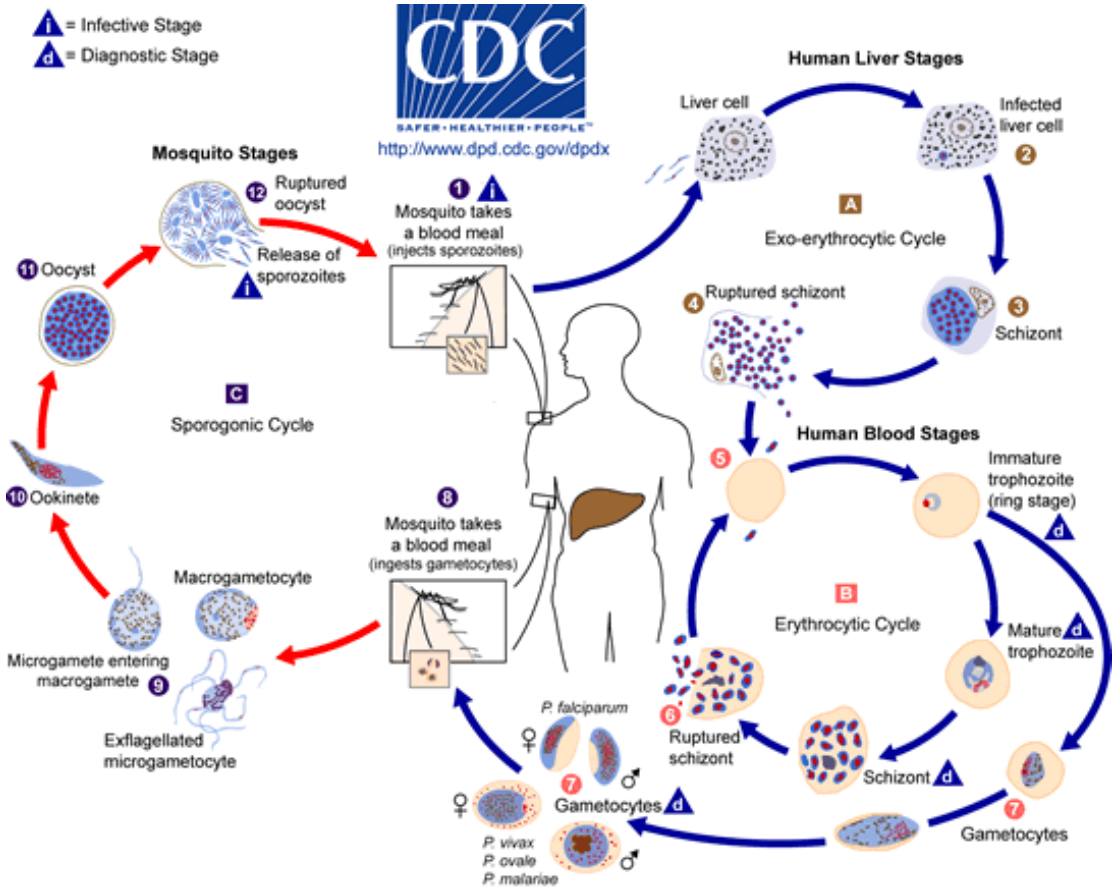


Figure 1.1. Transmission of the malaria *Plasmodium* parasite. This diagram shows the transmission cycle of the parasite from the *Anopheles* mosquito to the human host, including all known stages of the parasite's development within the host and the vector. Adapted from the Centers for Disease Control and Prevention, GA, USA (CDC 2010).

Not all merozoites develop into mature trophozoites within the red blood cells; some parasites do not divide but instead differentiate into microgametocytes (male gametocytes) and macrogametocytes (female gametocytes). The proportion of merozoites committing to developing into schizonts or into gametocytes depends on the stage of the *Plasmodium* infection (Bruce et al. 1990), and other factors such as development of an immune response, and possibly, seasonality of transmission.

The gametocytes are taken up by the female mosquito during her bloodmeal, and, once ingested migrate directly to the mosquito's midgut. Here they develop into microgametes (by exflagellation) and macrogametes respectively, and they merge, allowing the male gamete to fertilise the female gamete (Tuteja 2007). This sexual reproduction produces a zygote, which,

upon transformation into an ookinete, invades a cell of the mosquito's midgut lumen where it forms an oocyst. Within the oocyst, sporogony occurs, resulting in the production of thousands of sporozoites from a single oocyst.

Approximately 10-20% of the sporozoites generated in the oocyst reach the mosquito's salivary glands (the destination of the remaining 80-90% of sporozoites is unknown), from where they are injected into the human when the mosquito takes another bloodmeal (Hillyer et al. 2007).

2.3. Malaria control: vector-based interventions

The World Health Organization's policy of "integrated vector management" (IVM) to control malaria revolves principally around prevention by controlling the population of the *Anopheles* vectors, and reducing transmission, as well as by careful detection, treatment, and management of disease cases (WHO 2012a). This combination of strategies targets the transmission from vector to host (and from host to vector), and the development of the disease in humans (WHO 2014).

Vector-based control methods aim to reduce the local mosquito population in the vicinity of potential human hosts (mainly by reducing daily vector survival rates), and to decrease the probability of contact and bites between infective mosquitoes and humans (The malERA Consultative Group on Vector Control 2011; WHO 2014); this in turn is expected to lead to a reduction in the entomological inoculation rate (EIR), defined as the number of infective bites by mosquitoes per human per unit time (Kelly-Hope & McKenzie 2009), and thereby in local transmission rates.

The global strategy for vector control recommended by the WHO (WHO 1993), which highlights the importance of early diagnosis and immediate treatment of malaria cases, has been criticised as lacking evidence that this policy will reduce transmission (Shiff 2002). While this may help to prevent clinical malaria and clear existing infections faster, thereby removing the possibility for humans to transmit the infection to mosquitoes when bitten, it would be necessary to detect a very large proportion of cases to ensure interruption of transmission, which would be logistically impossible in remote areas in developing countries, where healthcare infrastructure is currently insufficient to reach the entire population.

2.3.1. Indoor residual spraying

Indoor residual spraying (IRS) is considered one of the most important methods of vector control currently in use, specifically in epidemic-prone areas (Roll Back Malaria 2005). It involves impregnating the walls and roofs of homes in a defined perimeter with a long-lasting chemical insecticide, of which the WHO has approved 12 for safe and effective use by indoor spraying (WHO 2006), with the aim of killing the mosquitoes that land and rest on the sprayed surface. This method protects a home for approximately 4-10 months (USAID 2011; WHO 2006).

The implementation of IRS in a given region, as well as the choice of insecticide to use within that region, will be affected by various factors, including the material of which the houses are made; the sensitivity of mosquitoes in that area to the insecticides under consideration; the duration of the malaria transmission period; and the legal status of the insecticides in the country (Pluess et al. 1996; USAID 2011). The effectiveness of IRS also depends on the biting and resting habits of the local species of *Anopheles* mosquitoes. IRS will have the largest impact on populations of endophagic (with a preference for biting indoors) and endophilic (with a preference for resting indoors) mosquitoes, as the more time the mosquitoes spend indoors the more likely they are to be affected by the insecticide (Pates & Curtis 2005). Drawbacks to IRS involve mainly the operational and logistical issues associated with the large teams needed to deliver insecticide to significant areas, and the emergence of resistance to insecticides by mosquito populations (Guyatt et al. 2002). In addition to this, practical considerations such as the correct dosage of the insecticide on the walls, and the timing of the spraying can considerably impact the success of the spraying campaign (Worrall et al. 2007).

The implementation of IRS has been shown to have a very important impact on the parasite rates (prevalence of infection) in young children (Fig. 1.2), as well as on the number of cases of malaria and hospital admissions due to malaria, within the targeted area, in particular within the first few years after its introduction (Mabaso et al. 2004). IRS has been estimated to reduce the risk of infection in all age groups by up to 75% (Guyatt et al. 2002; Pluess et al. 1996). It has also been shown to have an effect on the population structure of local *Anopheles* populations.

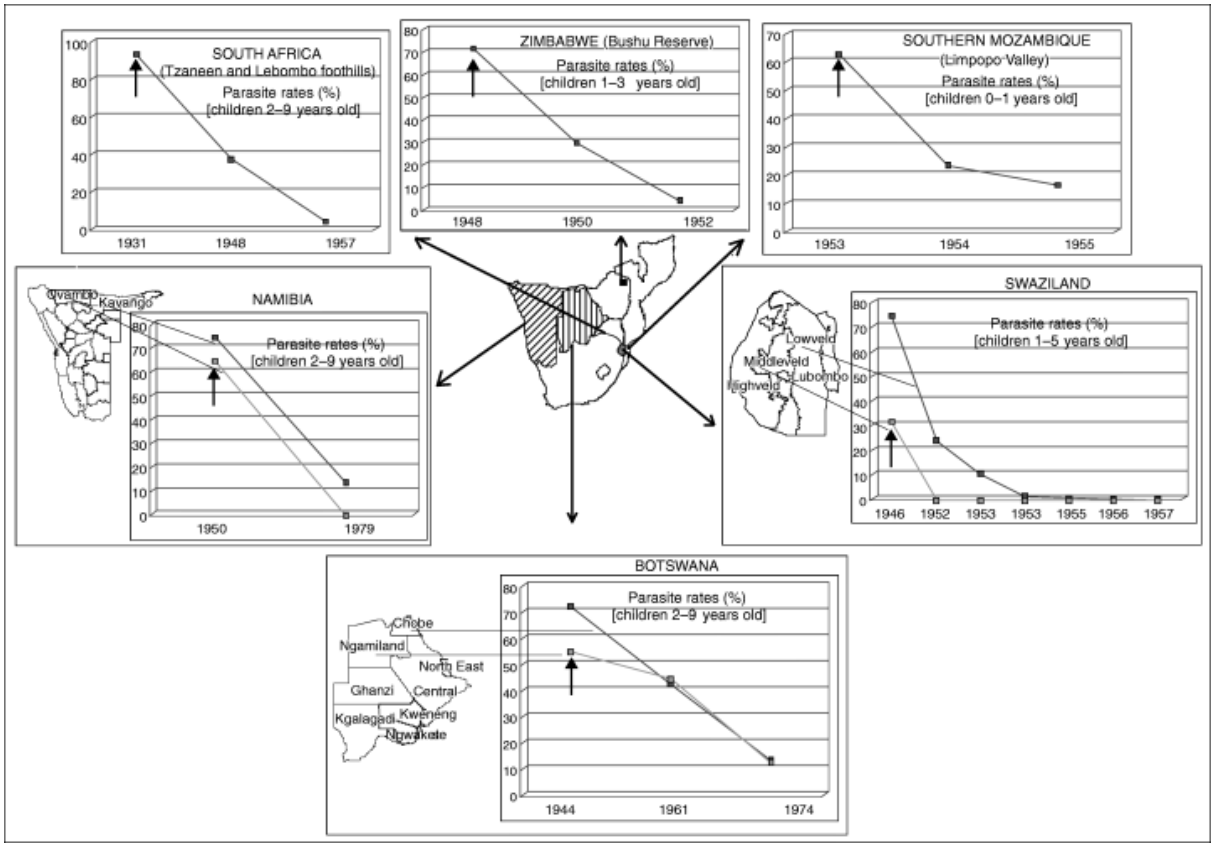


Figure 1.2. The impact of IRS. This shows the effect of IRS on parasite rates in years immediately following IRS introduction in 3 sub-Saharan African countries in which IRS has proven successful. The arrows indicate the year of implementation of IRS. The years immediately after the implementation of IRS show a dramatic reduction in parasitaemia in young children. Source: (Mabaso et al. 2004).

More endophilic and endophagic species, or species with a strong anthropophagic preference (the preference to feed on humans), such as *Anopheles gambiae* s.s., will be disproportionately affected, and may even be driven to localised extinction, while IRS may not have any effect on other species with more flexibly zoophagic or exophilic preferences, such as *Anopheles arabiensis* (Mabaso et al. 2004). This implies that the introduction of IRS, which aims to deter mosquitoes from entering the homes (Hamusse et al. 2012) and to kill endophilic female mosquitoes (Pluess et al. 1996) while having a considerable impact on the local vector populations, will not necessarily result in local elimination of malaria in areas with a highly heterogeneous population species composition, as transmission may be assured by other mosquito species.

2.3.2. Insecticide-treated nets, long-lasting insecticidal nets

ITNs and long-lasting insecticidal nets (LLINs) have become very important instruments and are considered, along with IRS, one of the two main methods of malaria prevention in highly

endemic areas (WHO 2008). As *Anopheles* mosquitoes prefer as a rule to feed on humans during the night (Mbogo et al. 1993), sleeping under LLINs reduces the possibility of contact between mosquitoes and humans, and therefore the possibility of biting (Fig. 1.3).

It has been suggested that the main effect of LLINs is to reduce transmission intensity by decreasing the lifespan of mosquitoes when they rest on the insecticide-impregnated net (killing by knock-down effect), by lengthening the time between bloodmeals (diverting mosquitoes to other, unprotected hosts), and by diverting at least a proportion of bites onto non-human hosts by making contact with humans difficult (LeMenach et al. 2007). The WHO has recommended using exclusively LLINs (WHO 2008) as the fabrication process allows the insecticide to resist frequent washing, avoiding the need to re-impregnate as in classical ITNs. There exist several types of LLINs, impregnated with different insecticides or combination of insecticides (mainly synthetic pyrethroids), for use in different settings and in case of emergence of resistance to any one insecticide (Lindblade et al. 2005).

The use of ITNs and LLINs is strongly encouraged either alone or in combination with IRS, as bednets are extremely cost effective (Goodman & Mills 1999; Wiseman et al. 2003), easy to use and require little technical expertise to implement (Binka & Akweongo 2006), and provide very good protection to the individuals sleeping under them (Chinnock 1999).



Figure 1.3. Insecticide-impregnated bed nets. Insecticide-treated nets are soaked in or impregnated with an insecticide, and need to be re-treated after 3 washes, whereas long-lasting insecticidal nets are made from netting material that has insecticide incorporated within the fibres, and can retain its insecticidal quality for up to 3 years (WHO 2008).

One of the main challenges in the implementation of an effective LLINs policy is attaining the necessary population-wide coverage in a malaria-endemic country (Binka & Akweongo 2006; Nahlen et al. 2003). While IRS provides a population-wide benefit by decreasing the vector population, ITNs and LLINs profit the individuals sleeping under them with little added benefit at the community- or even household-level (Nahlen et al. 2003), although they may succeed in reducing the number of mosquitoes in the room in which they are deployed

(Chinnock 1999). This implies that widespread use of bednets is necessary to effectively reduce malaria transmission (Birget & Koella 2015).

Vector control techniques may provide a feasible method of reducing transmission. Implementation of ITNs and LLINs has been observed to reduce child mortality and morbidity due to malaria between 17% and 27%, and to reduce the probability of a child dying before its fifth birthday by 29%, provided a high coverage is maintained (Binka & Akweongo 2006; Lengeler 2004). It has been estimated that IRS reduces malaria incidence by up to 54% in regions with endemic malaria and by up to 93% in areas of epidemic malaria (Pluess et al. 1996), and ITNs reduce the incidence of malaria cases by up to 50% in areas of endemic malaria, and by up to 62% in areas of epidemic malaria (Lengeler 2009); the use of ITNs when combined with IRS has a protective efficacy (PE) against malaria-related mortality of approximately 55% (Eisele et al. 2010). The introduction of larvicides has been shown to reduce transmission, as measured by the EIR, by up to 32% and thereby to reduce the risk of malaria in young children by up to 72% (Geissbühler et al. 2009).
This suggests that antivectorial methods can be efficient in reducing the prevalence and incidence of malaria, provided they are applied on a sufficiently large scale and used correctly, and provided the mosquito does not develop resistance to the pesticides.

2.3.3. Other methods

Besides IRS and bed nets, there exist other intervention methods aiming to control the vector population, used mainly in conjunction with the two techniques described above. Larval control, by environmental management and with the use of larvicides, may be efficient in reducing the vector population in the vicinity of human populations. Environmental management is defined by the WHO as consisting of environmental modification ("permanent infrastructural changes of a capital-intensive nature") and environmental manipulation ("recurrent actions aimed at achieving temporary unfavourable conditions for vector breeding"), and relies on constant monitoring of potential breeding sites (WHO 2012b). This involves altering the water landscape where possible (such as draining ponds or stagnant water bodies), covering potential breeding sites (such as water tanks or latrines), or placing non-toxic chemicals or microbial agents into the water (DFID 2010). However, environmental management and larvicide introduction is only effective in areas with relatively few, fixed, and contained breeding sites in order to be able to target them specifically. For this reason, environmental management, and the use of larvicides in breeding sites, are well suited to

urban settings, in which water bodies are usually smaller and well defined, but are rarely cost-effective in rural settings (Thwing et al. 2011). In such rural environments with large bodies of water, introducing larvivorous fish may help to reduce the vector population, although there is as yet no evidence of an impact on malaria transmission (DFID 2010).

Late-life acting biopesticides are a relatively new method, designed to minimise the possible emergence of resistance. This method is intended to allow young adult mosquitoes to survive and lay eggs, which reduces the selection pressure for resistance (Koella et al. 2009a; Koella et al. 2009b; Read et al. 2009). The success of such biopesticides depends on their ability to kill the mosquito early enough to break the transmission chain, yet late enough not to endanger the survival of the species, thereby slowing down the evolutionary need for resistance (Lorentz & Koella 2011). Entomopathogenic fungi (Blanford et al. 2011; Hancock et al. 2009; Scholte et al. 2005; Thomas & Read 2007) and microsporidia (Koella et al. 2009) are two agents currently being investigated as possible biopesticides.

The sterile insect technique (SIT) relies on the release of transgenic (Benedict & Robinson 2003) or irradiated (Helinski et al. 2008) male mosquitoes into a localised population of vectors. The success of this method is logistically challenging, as it requires vast numbers of adult mosquitoes to be released over a period of years, and relies on preventing immigration of mosquitoes from surrounding areas from migrating into the SIT-delimited region (Lofgren et al. 1974), and has the disadvantage of being species-specific (Benedict & Robinson 2003), whereas other methods of vector control may impact other insect vectors. However, without an influx of external wild-type mosquitoes, as the vector population begins to decline the impact of the SIT increases, increasing its efficacy in preventing malaria transmission, and this method has the additional advantage of being environmentally benign (Benedict & Robinson 2003).
Transgenic mosquitoes may also be designed and bred to be resistant to malaria, in the hope they may pass down the resistant gene to their progeny (DFID 2010).

2.4. Malaria control: other interventions

As well as vector control, other interventions aim to reduce the burden of malaria at various phases of the transmission cycle. These include vaccines, which can target different stages of the parasite's lifecycle (Fig. 1.4, Alde et al. 2007; Miller et al. 2002); prophylaxis (Eisele et al. 2010), or drugs such as ivermectin, which although ingested by humans (as anthelminthic

treatment against filarial and soil-transmitted nematodes) kill the mosquitoes when they bite (Chaccour et al. 2010).

The pre-erythrocytic vaccines (PEVs) target the liver stages of the parasite in the human by directing an immune response against the sporozoites or intra-hepatocytic stages, and aim to induce immunity to clear the infection before the parasite reaches the bloodstream so as to prevent clinical symptoms (Alde et al. 2007; Hill 2005; Nardin et al. 1999).

Other types of potential vaccines include blood-stage, or erythrocytic, vaccines (EVs), which aim to prevent the invasion of red blood cells by the parasites thereby decreasing the severity of clinical symptoms, but not blocking transmission (Alde et al. 2007), and transmission-blocking vaccines (TBVs), which target the parasite's stages inside the mosquito by inducing human antibodies to prevent establishment of the infection within the mosquito after it bites an infected human, thereby blocking transmission from humans to vectors (Alde et al. 2007; Carter 2001; Kaslow et al. 1988). Vaccines may prove effective in helping to interrupt transmission: the anti-sporozoite vaccine RTS,S has been shown to have a PE of 60-80% (Abdulla et al. 2008; Bejon et al. 2008; Penny et al. 2015). With a wide enough coverage, it is expected that this could be a crucial tool in preventing transmission from humans to the vector, thereby considerably influencing the spread of malaria.

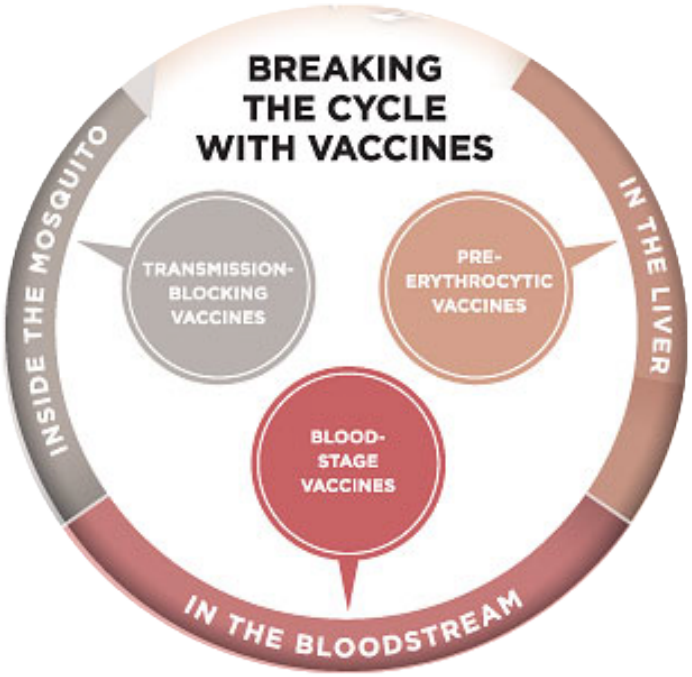


Figure 1.4. The stages targeted by potential malaria vaccines. Malaria vaccines can target any of the phases of the *Plasmodium* lifecycle: the pre-erythrocytic, or hepatic stages, during which the parasite reproduces asexually; the erythrocytic, or blood stages, during which the parasite reproduces also asexually; and the mosquito stages, where there is sexual reproduction. (Alde et al. 2007). Figure from (PATH 2012).

3. Malaria vectors

3.1. Species of *Anopheles* mosquitoes transmitting human malaria

The mosquito genus *Anopheles* (Diptera: Culicidae) is the only vector currently able to transmit the *Plasmodium* parasite to humans, which is responsible for malaria. However, not all species of *Anopheles* are competent to transmit all species of *Plasmodium* infecting humans, and there exists a relatively exclusive specificity of an *Anopheles* species for a particular species of *Plasmodium* (Billingsley & Sinden 1997). Non-human malaria parasites may be transmitted by other mosquito genera.

There are currently between around 500 known species of *Anopheles* mosquitoes (Hay et al. 2010; WHO 2007). While 104 species are able to transmit human malaria, between 20 and 70 of these are considered major vectors of human malaria in endemic settings (Lal et al. 2001; Mohanty et al. 2007; WHO 2007). In Africa, where approximately 80% of all malaria cases and 91% of all malaria deaths occurred in 2010 (WHO 2011), the main vectors of human malaria are *An. gambiae* s.s., *An. arabiensis*, and *An. funestus* (Hay et al. 2010; Sinka et al. 2012; Tonnang et al. 2010), which transmit mainly *P falciparum*, and to a lesser extent *P. ovale* (Carter & Mendis 2002; Hay et al. 2010; Sinka et al. 2012).

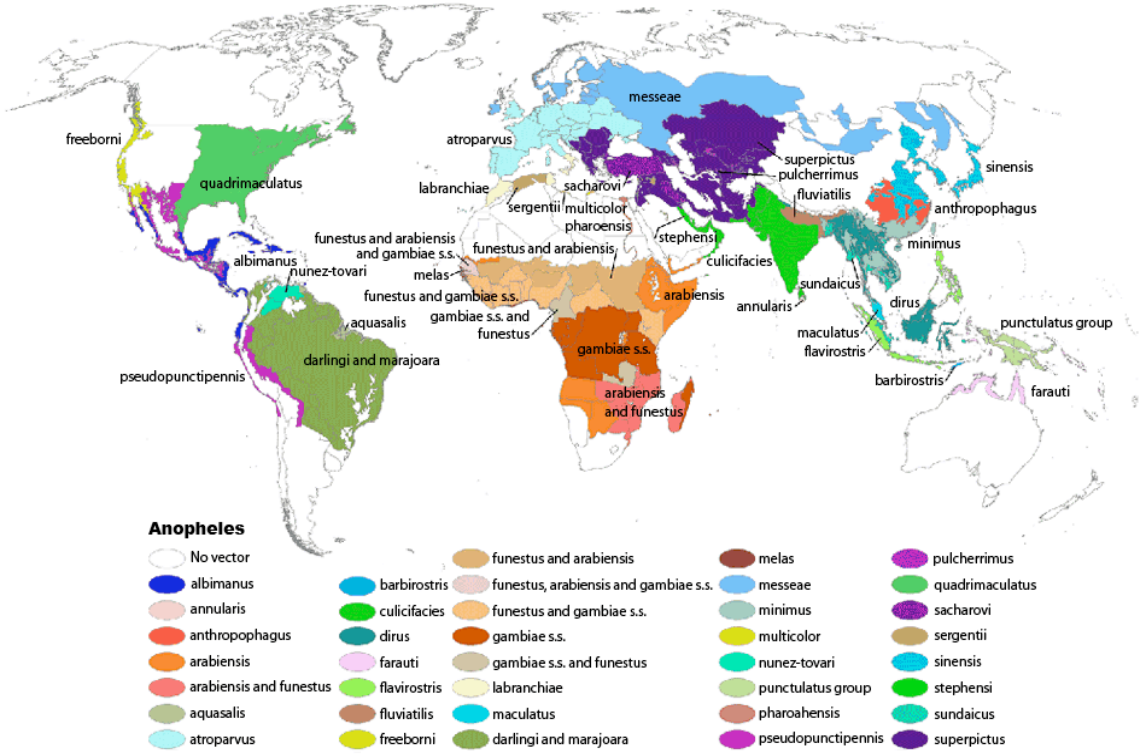


Figure 1.5. Worldwide distribution of *Anopheles* mosquitoes. Human malaria is transmitted by different *Anopheles* species depending on the geographical location. *Anopheles* species capable of transmitting malaria exist also in areas where malaria has been eliminated (anophelism without malaria), posing a risk of re-introduction if the environmental conditions are suitable for transmission (Sinka et al. 2012).

However, in South-East Asia, the second largest region in terms of malaria burden, where 13% of all malaria cases and 6% of all malaria deaths occurred in 2010 (WHO 2011), a greater number of *Anopheles* species are involved in the transmission of malaria, in addition to the three main species: *An. dirus*, *An. minimus*, and *An. sundaicus* (Hay et al. 2010; Sinka et al. 2012; Trung et al. 2005), which transmit mainly *P. vivax*, although they have recently been found to also transmit the emerging human malaria parasite, *P. knowlesi* (Kantele & Jokiranta 2011).

3.2. Biology and life-history of *Anopheles* mosquitoes

Although the *Anopheles* genus comprises over 440 individual species (Harbach 2004), the life history of these species is relatively similar.

The mosquito maturation process comprises three immature, aquatic stages and an adult stage (Fig. 1.6). The female *Anopheles* mosquito lays 50 to 200 eggs on a water body. Different species seek out specific characteristics in breeding sites in terms of water depth, size of water body, and nature of water body, i.e. stagnant or running water (Machault et al. 2009; Pinault & Hunter 2012). The eggs will hatch into larvae between 2 days and 3 weeks after oviposition, depending on the species and environmental factors (CDC 2010).

Larvae develop through four stages, or instars, and shed their exoskeleton between each phase, before metamorphosing into pupae. The first two stages last approximately 24 hours each, and the last two stages approximately 36 hours, although this depends on environmental factors, and males tend to develop faster than females (Benedict 2007). Throughout these instars, larvae live in the water, positioning themselves parallel to the surface in order to breathe, and diving below the surface only as an escape reflex. Larvae feed on algae, microorganisms, and bacteria present in the organic microlayer at the water surface, depending on the habitat (CDC 2010), although fish food has been found to fulfil their dietary requirements in the laboratory (Benedict 2007).

The duration of the pupal stage is short and usually lasts a couple of days. Similarly to larvae, pupae remain close to the water surface to breathe, and also possess the dive reflex to escape potential predators (CDC 2010), though they do not feed. While male *Anopheles* pupae are smaller than female pupae, this difference is too subtle to be observable with the naked eye (Benedict 2007).

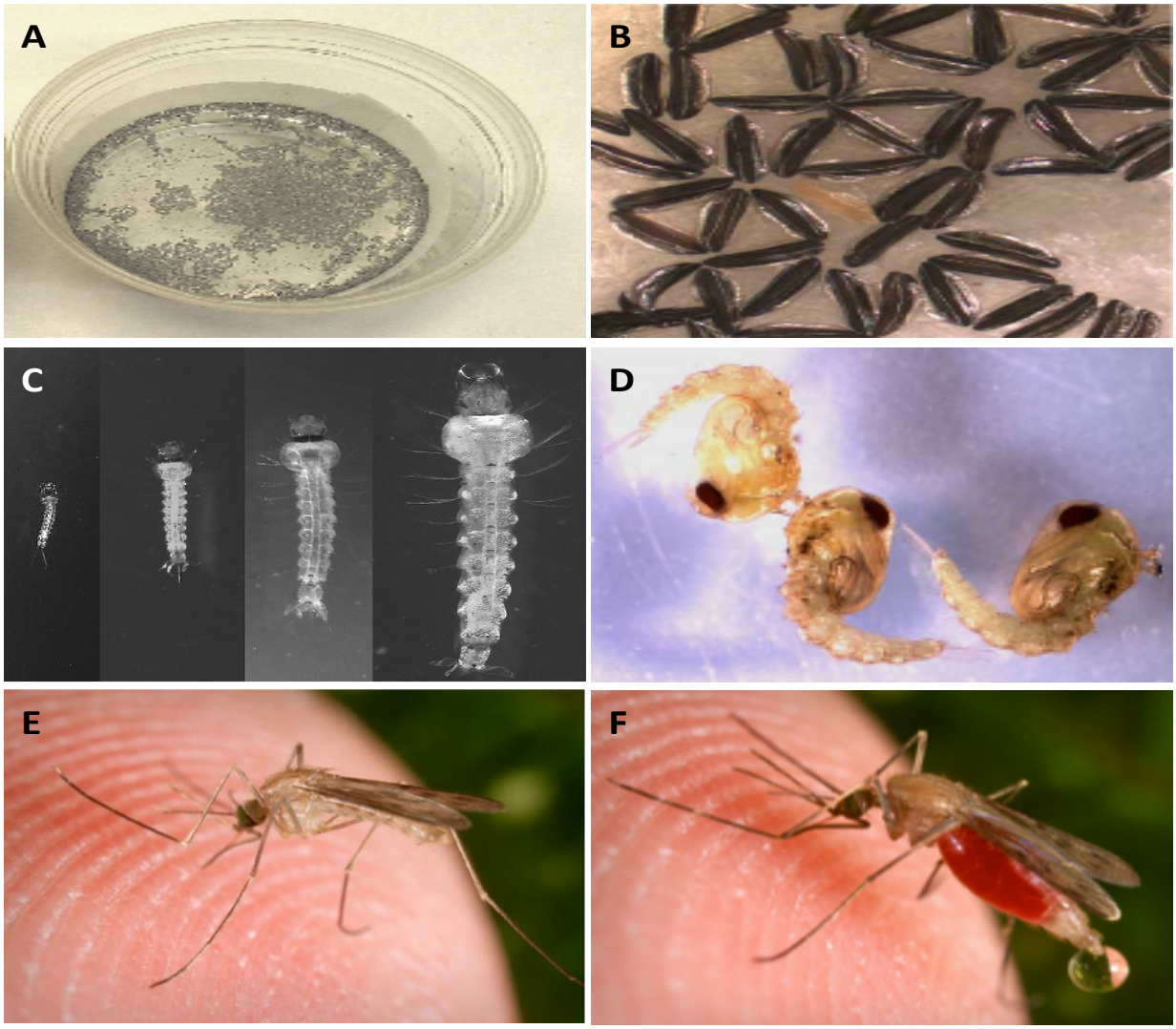


Figure 1.6. The life stages of the *Anopheles* mosquito. A, *Anopheles gambiae* s.s. eggs laid by many females in a small water bowl. **B**, close-up of the eggs shown in A. **C**, *Anopheles funestus* s.s. larvae of stages L1, L2, L3, and L4, from left to right, photographed at the same magnification. **D**, *Anopheles gambiae* s.s. pupae (Benedict 2007). **E** and **F** respectively, adult *Anopheles gambiae* s.s. mosquito pre (E) and post (F) bloodmeal, the abdomen swells and engorges once the female begins pumping in blood and excreting unwanted liquid to concentrate the blood protein, as seen in F (CDC 2010).

The time from egg to emergence as an adult can range from 5 to 14 days depending on environmental conditions. Male *Anopheles* live for approximately a week, feeding on a diet of sucrose, fructose, or glucose, and mating occurs typically 1-2 days after emergence, although this can vary widely depending on population density; the female then requires a bloodmeal to produce a batch of eggs, and after feeding will rest while digesting the blood and developing the eggs. This cycle of blood-feeding and laying eggs (gonotrophic cycle) will continue until the mosquito's death, which in the natural environment occurs, on average, 1 to 2 weeks after emergence from pupae (Clements & Paterson 1981; CDC 2010).

4. Current data on temperature-dependent mosquito life-history parameters

4.1. Effect of temperature on *Anopheles gambiae* life-history parameters

As with other cold-blooded disease vectors, *Anopheles gambiae* is sensitive to environmental temperature and its changes, which have been shown to impact the survival of eggs (Depinay et al. 2004; Gonçalvez de Carvalho et al. 2002; Horsfall 1972; Huang et al. 2006; Impoinvil et al. 2007), larvae (Afrane et al. 2007; Bayoh and Lindsay 2004; Bayoh and Lindsay 2003; Kirby and Lindsay 2009; Lyimo et al. 1992), pupae (Armstrong & Bransby-Williams 1961; Depinay et al. 2004), and adult mosquitoes (Kirby & Lindsay 2004; Afrane et al. 2006; Afrane et al. 2007a; Midega et al. 2007; Olayemi & Ande 2008; Lunde et al. 2013), as well as the duration of the gonotrophic cycle (Rúa et al. 2005).

In order to predict global transmission patterns, crucial parameters defining the sensitivity of mosquitoes and mosquito-parasite interactions to environmental factors must first be quantified (Rogers & Randolph 2000). While there is some evidence as to temperature-dependent survival of *Anopheles* mosquitoes, very little has been done towards quantifying other characteristics of the vector and the vector-parasite interactions in relation to climatic and other variables. It is also hypothesised that factors such as temperature, humidity, and availability of food will influence not only mosquito survival, but also developmental rates across all stages, which in turn will impact a mosquito's life-history parameters and competence as a vector. For instance, there are currently few data on how temperature affects larval developmental rates, or on its impact on adult life-history traits such as size, fecundity, bloodmeal size, and competence for the malaria parasite as a vector (Craig et al. 1999; Horsfall 1972; Jepson et al. 1947; Lyimo et al. 1992; Lyimo & Takken 1993; Takken et al. 2013; Ng'habi et al. 2005; Takken et al. 1998b; Paaijmans et al. 2010). Given that various tools used in the push to eliminate malaria, including interventions aiming to reduce vector abundance and model predictions of vector population dynamics, rely heavily on data to inform them, this lack of comprehensive and precise data leads to considerable uncertainty around the impact of interventions, or the reliability and robustness of modelling forecasts. As such, detailed experimental studies of the malaria vector, both in the laboratory and in the field, are of the highest importance to inform theoretical studies and policy.

4.2. Effect of temperature on *Anopheles gambiae* geographical distribution

The sensitivity of malaria vectors to temperature minima and maxima has been one of the key factors determining current distribution of malaria worldwide (Hay et al. 2004; Ermert et al. 2012). Experimental data consistently show that there is a minimum temperature around 5°C (Bayoh and Lindsay, unpublished), and a maximum temperature around 40°C (Huang et al.

2006; Kirby & Lindsay 2004), respectively below and above which, *Anopheles gambiae* mosquitoes cannot survive. Conversely, in areas close to the physiological tolerance of mosquitoes, a very small increase in temperature can lead to a substantial increase in the number of mosquitoes in that region (Patz & Olson 2006). Whereas the geographical distribution of mosquitoes is species-specific (Moffett et al. 2007), and some species have adapted to colder temperatures, the combination of malaria transmission-competent *Anopheles* and climates in which the *Plasmodium* parasites thrive is highly dependent on geographical compatibility. In addition, it has been demonstrated that higher environmental temperatures correlate with higher *Anopheles* abundance (Obala et al. 2012; Bashar & Tuno 2014; Juri et al. 2014; Munhenga et al. 2014). Higher temperatures have also been shown to result in an increase in malaria prevalence and incidence (Kim et al. 2012; Friedrich 2014; Guo et al. 2015), although the effect of temperature fluctuations on malaria incidence appears to depend on the baseline environmental temperature around which the fluctuations occur (Blanford et al. 2013; Zhao et al. 2014).

5. Other factors influencing mosquito life-history parameters

5.1. Wind and rainfall

Besides temperature, three other main climatic factors are believed to influence malaria transmission significantly, two of which are wind and rainfall. Wind can be expected to impact the spread of malaria by influencing the dispersal patterns of the mosquito vector and the viability of breeding sites by determining evaporation rates (Parham et al. 2011).

The impact of rainfall on malaria transmission is manifested in the creation and modification of breeding sites. The immediate effect of increased precipitation is to increase the number of water bodies and therefore of potential habitats: this is supported by evidence that *Anopheles* abundance increases with bouts of rainfall in dry areas, albeit with a certain time lag, which suggests that precipitation may predict vector abundance patterns. This rainfall-induced increase in vector abundance may also have repercussions for disease transmission, as heavy rainfall leading to higher mosquito abundance may correlate with malaria incidence (Pascual et al. 2008; Galardo et al. 2009; Krefis et al. 2011; Silal 2012; Wardrop et al. 2013). On the other hand, excessive rain, or rainfall in areas with already regular precipitation, can have the opposite effect, by flooding previous breeding sites, and thereby increasing the mortality rate of *Anopheles* immature stages (Paaijmans et al. 2007; Barbosa et al. 2014). In addition, there is some evidence suggesting that certain mosquitoes prefer wetter areas, and certain species of

Anopheles are more abundant in areas with regular or heavier rainfall (Lamidi 2009; Naranjo-Diaz et al. 2013).

5.2. Humidity

The fourth major climate-related factor that can influence malaria transmission is relative humidity (RH), which affects adult *Anopheles* mosquitoes' survival, activity, and reproduction. As with temperature, there appears to be a range of relative humidity within which mosquitoes' life-history parameters are at their maximum. At RH below 60%, and close to 100%, mosquito survival is significantly shorter ; on the other hand, as humidity increases within these ranges, conditions are favourable for mosquito reproduction, leading to a larger vector population (Jawara et al. 2008).

RH levels are expected to remain comparatively constant under climate predictions, although there are considerable daily variations on small regional scales (Willett et al. 2008), and current research is aiming to establish the relationship between RH and malaria transmission (Tian et al. 2008; Haque et al. 2010; Huang et al. 2011; Kim et al. 2012).

5.3. Density-dependence and food availability

Mosquitoes are also sensitive to other environmental factors, including crowding during the development of the immature stages. This has been demonstrated in laboratory conditions, where survival significantly depends on the density of larvae in the water body. Beyond a certain number of larvae per millilitre of water, the survival probability decreases by up to 60% (Gimnig et al. 2002; Koenraadt & Takken 2003; Koenraadt et al. 2004; Ng'habi et al. 2005; Timmermann & Briegel 1993; Tsila et al. 2011). This larval density-dependent survival is due to competition for food as well as over-crowding, and has other implications for the following developmental stages. It is recognised that adult *Anopheles* that experienced crowded conditions as larvae are smaller, have longer development times, reduced pupation and eclosion rates, and decreased pupal weight (Benedict 2007), although these features have currently not been quantified.

It is thought, however, that under natural conditions density dependence will occur to a much lesser extent, as predation on the immature stages will considerably diminish the density of the larvae in the breeding sites, and adult female, gravid mosquitoes may tend to oviposit in less crowded breeding sites (Munga et al. 2006).

5.4. Anthropogenic change (climate change and antivectorial interventions)

In addition, because of the sensitivity of both the *Anopheles* vector and *Plasmodium* parasite to temperature, and the sensitivity of the vector to rainfall, wind, and humidity, the distribution of the mosquito and therefore of malaria is expected to be influenced by climate, and any changes in climate will have an effect on mosquito localisation and thereby on malaria transmission (Lindsay & Birley 1996; Lauderdale et al. 2014; Tay et al. 2012). It is thought that in regions currently favourable for malaria transmission, or with already stable transmission, where vector population dynamics are relatively stable, climate change is likely to have relatively little effect on malaria in the African lowlands (Lindsay & Birley 1996; Gething et al. 2010; International Panel on Climate Change (IPCC) 2013), whereas it will likely affect transmission in highland areas to a much greater extent (Hay et al. 2002; Koenraadt & Githeko 2005; Siraj et al. 2014; Caminade et al. 2014; Ermert et al. 2012).

6. Mathematical models of malaria vector population dynamics

Mathematical modelling is an extremely valuable instrument, allowing an accurate representation of a complex system such as the effect of interactions between biotic and abiotic factors on malaria transmission, which can lead to a better understanding of the disease's epidemiological patterns. This enables more accurate targeting of elimination efforts, and more realistic predictions of the outcome of interventions (Eckhoff et al. 2014; Smith et al. 2006). In modelling VBDs, it is necessary to account for the role played by the vector in the transmission of the disease, either implicitly, or by explicitly including a vector component in the model (Reiner et al. 2013).

Models that wish to explore the manner in which the *Anopheles* mosquitoes are responsible for the spread of malaria, and how intrinsic mosquito characteristics and population dynamics affect the parasite's lifecycle, may need to model the vector component in greater detail. This involves modelling the mosquito population dynamics as efficiently as possible, such as including intra- and inter-species competition, predation, and density-dependent effects (Kirby & Lindsay 2009; Paaijmans et al. 2009), and/or age-dependent survival probabilities (Churcher et al. 2010; Dawes et al. 2009; Hancock et al. 2009; Hancock & Godfray 2007).

Such detailed modelling relies on field and experimental evidence, in order to quantify relevant parameters and their associated uncertainty, but the quality and quantity of data available are limited, due to the difficulty of recreating field conditions in the laboratory

(Parham et al. 2011; Service 1993), and the intrinsic differences between *Anopheles* species and even between habitats for the same species (Juliano 2007).

In the case where the influence of external factors (such as climatic variables or density-dependence) on malaria vectors is of relevance (Hoshen & Morse 2004; Depinay et al. 2004; Parham et al. 2012; Lutambi et al. 2013), or where different mosquito stages may be affected differently by external factors (such as in the case of interventions targeting specific developmental stages) (White et al. 2011; Arifin et al. 2014; Patinvoh & Susu 2014; Chitnis et al. 2010) it is useful to model explicitly the vector component, and sometimes even the different developmental stages of the mosquito. As a branch of entomological research is currently focusing on defining the influence of climate change and global warming specifically on malaria-transmitting mosquitoes, more models depicting solely the population dynamics of the vector are being developed, focusing on rendering the details of the complex biological, entomological, and ecological mechanisms of the mosquito population as realistically as possible (Rogers 1994; Struchiner et al. 2006). Vector models can also give valuable insights into the infectious potential of a mosquito population, without necessarily including a transmission element (Arifin et al. 2014; Beck-Johnson et al. 2013; Patinvoh & Susu 2014; Chitnis et al. 2010).

As the importance of environmental factors in the spread of malaria is recognised (Gething et al. 2011), more mathematical models of vector population dynamics have begun to include the impact of climate-related factors on the mosquito's life-history parameters (Hoshen & Morse 2004; Jones & Morse 2010; Lindsay & Martens 1998; Morse et al. 2005; Paaijmans et al. 2012; Paaijmans et al. 2010; Paaijmans et al. 2008; Parham & Michael 2010; Beck-Johnson et al. 2013). Vector population dynamics and abundance impact the transmission dynamics of the disease the vector transmits (Martens et al. 1995; Martens et al. 2002; Charron et al. 2013; Roiz et al. 2014), so the effect of climatic variables on *Anopheles* life-history traits indirectly affects malaria transmission also. However, while including environmental variables in transmission models aims to enhance the realism and relevance of the models' output, modelling the interactions between environmental factors and malaria transmission will append different types of uncertainty to the model, until sufficiently accurate experimental data are available to parameterise the climate-related sections of the model (Parham et al. 2011).

As indicated in section 4.1 of this chapter, there is currently insufficient comprehensive data on the effect of temperature on *Anopheles* life-history parameters and this has a trickle-down

influence on modelling studies. Reiner et al. (2013) point out that many of the common assumptions in modelling studies have remained unchallenged for a number of years, particularly as regards model parameterisation.

7. Research Objectives

The overall aim of this thesis was to gather experimental data that would be used to inform and develop a biologically realistic model of *Anopheles gambiae* population dynamics.

The primary objectives included

- To investigate experimentally the effect of environmental temperature during all life stages of *Anopheles gambiae* s.s. on survival
- To determine whether senescence happens in the mosquito (i.e. whether age affects survival)
- To quantify, experimentally, the effect of environmental temperature on other life-history parameters relevant for reproduction and population dynamics
- To develop a mathematical model of *Anopheles gambiae* s.s. abundance informed by and based on the experimental data
- To fit the model to longitudinal abundance data

CHAPTER 2

THE EFFECT OF ENVIRONMENTAL TEMPERATURE DURING LARVAL AND ADULT STAGES ON *ANOPHELES GAMBIAE* S.S. LARVAL AND ADULT SURVIVAL

1. Introduction

Historical data and theoretical models indicate that the geographical distribution of malaria is very sensitive to the implementation of control measures as well as to climatic factors, nonetheless it appears evident that climate affects the distribution of insect disease vectors such as mosquitoes (Afrane et al. 2012; Roiz et al. 2014; Siraj et al. 2014) and thereby influence the transmission of VBDs such as malaria, and the extent to which these diseases scan be controlled. However, we currently have a limited understanding of how and to what extent climatic factors influence entomological parameters determining transmission. The question most obviously relevant to disease transmission is how changing temperatures will affect mosquito longevity and the duration of the parasite's development within the mosquito, as these are two of the most important parameters affecting mosquito-borne disease transmission (Lambrechts et al. 2011; Adelman et al. 2015).

However, temperature also influences mosquito life-history traits connected with vector competence and population density: warmer conditions may lead to faster development and smaller adults (le Sueur & Sharp 1991; Takken et al. 1998b; Charlwood & Bragança 2012). The size of the adult mosquito can influence epidemiologically relevant traits including longevity, the length of the gonotrophic cycle (and thus the probability of a female mosquito laying eggs before dying), immunocompetence, blood meal size (and thereby probability of infection), biting rates, and intensity of infection (Lyimo & Takken 1993; Takken et al. 1998b). These parameters can in turn affect mosquito survival (Dawes et al. 2009) and the parasite development in the mosquito (Churcher et al. 2013). The effect of temperature on the mosquito life history may also indirectly influence transmission through fecundity, as this is limited by size. In addition, mosquito population density and fecundity affect larval density, and thereby influence the development of mosquitoes through larval density-dependent competition and mortality (White et al. 2011). It has also been suggested that the maternal environment has an influence on the population dynamics of *Anopheles* mosquitoes through its impact on offspring development, survival, and susceptibility (Vogels et al. 2014).

Although sketches of these interactions have been outlined, a detailed picture of their integration is missing but needed to allow more reliable predictions of how temperature may influence malaria transmission. Malaria is transmitted to humans through the bite of female *Anopheles* mosquitoes. In order to acquire and transmit the infection, mosquitoes must bite at least twice. The *Plasmodium* parasites then undertakes its complex sporogonic cycle within the vector, and depending on environmental temperatures, the extrinsic incubation period duration can be similar to the mosquito's average life expectancy (Bellan 2010). It is therefore necessary for the mosquito to survive until sporogony has been completed and beyond, in order to transmit the sporozoites to a host: this makes malaria transmission especially sensitive to the daily survival probability of the vector.

Anopheles mosquitoes are affected both by the mean temperature of their environment and by its temporal fluctuations (Paaijmans et al. 2009; Paaijmans et al. 2010; Paaijmans et al. 2013). Understanding the influence of temperature on mosquito ecology is therefore valuable in predicting vector distribution, fitness, and capacity to transmit malaria (Semenza & Menne 2009). As environmental shifts due to climate change are likely to affect the global spread of malaria (Sutherst 2004), and climatic factors that influence vector survival are particularly likely to influence malaria transmission (Craig et al. 1999), an insight into the factors affecting mosquito populations will also improve predictions of malaria transmission. Currently the extent to which the vector population dynamics depend on climatic factors is still under investigation (Sutherst 2004; Craig et al. 1999; Lafferty 2009; Beck-Johnson et al. 2013).

Mosquito survival has already been shown to be subject to temperature, rainfall, and humidity (Warrell & Gilles 2002; Yamana & Eltahir 2013), as well as other influences including mosquito density (Gilles et al. 2011; Muriu et al. 2013), genetic diversity (Tchuinkam et al. 2010), and the availability of blood meals. Several studies have looked into the influence of environmental temperature during the adult stage on adult survival (Afrane et al. 2007b; Afrane et al. 2006; Kirby & Lindsay 2004; Midega et al. 2007; Olayemi & Ande 2008), but fewer data exist on the effect of the immature environmental temperature on the survival of immature mosquitoes (Bayoh & Lindsay 2004; Bayoh & Lindsay 2003; Huang et al. 2006; Impoinvil et al. 2007; Kirby & Lindsay 2009). There are currently no data on the effect of temperature of the immature stages' environment on adult mortality, although temperature throughout the mosquito's development may affect its survival at all stages (Beck-Johnson et al. 2013).

This chapter presents the results of an experiment investigating the effect of environmental temperatures during *An. gambiae* s.s. immature and adult stages on survival at the larval and adult stages.

2. Methods

2.1. Larval maintenance and temperature regimes

Anopheles gambiae sensu stricto (*s.s.*) mosquitoes, originally from the Kisumu colony in Western Kenya, are continually maintained in a colony at Imperial College London's Silwood Park campus at a constant temperature of 26°C (±2°C), with relative humidity set at 70% (±10%) and a 12/12 light/dark photoperiod. Colony larvae are reared 150 larvae to a basin (45cm x 30cm x 10cm) in 2-3cm of distilled water, and fed daily with a pinch of TetraMin™ fish food. Any pupae are removed every day and placed in distilled water in a 250ml cup in a plastic cage (45cm x 45cm x 45cm, Fig. 2.1, A) to emerge into adults. A 100ml cup containing Whatman™ filter paper partly immersed in a saturated sugar solution (10% sugar in distilled water) is placed in the cages as a food source for the adult mosquitoes (Fig. 2.1, B). Approximately 4-5 days after emergence, and once a week thereafter, the adult mosquitoes are given a blood meal and a 250ml cup filled with distilled water is placed in the cages to allow the females to lay eggs.

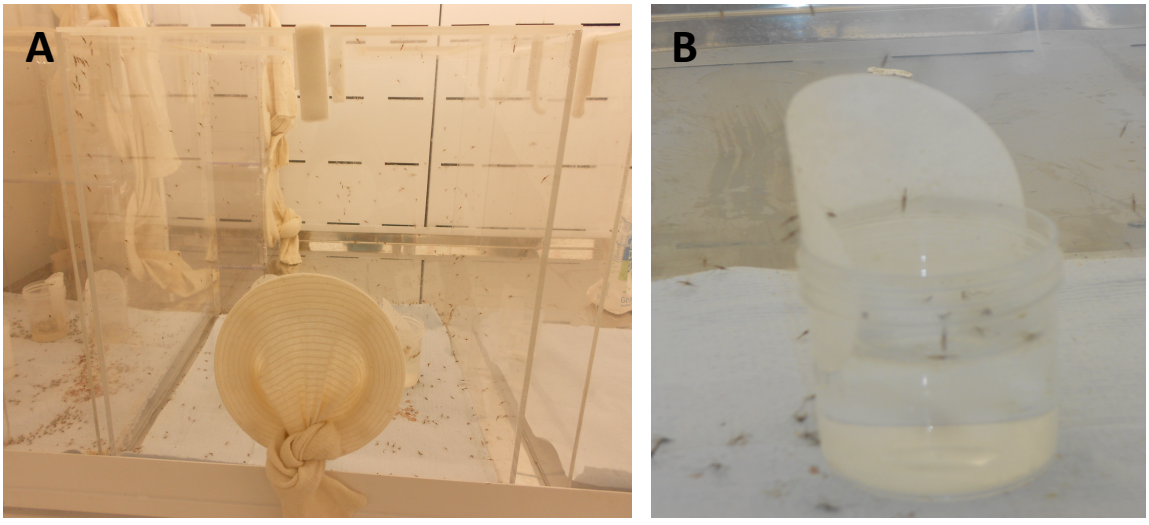


Figure 2.1. Cages and food source cups. **A**, cages in which adult mosquitoes are kept at Silwood Park. The sleeve at the front of the cage allows the researcher to reach into the cage to place emerging pupae or sugar water inside, and to remove eggs or individual mosquitoes. **B**, a 250ml cup containing the sugar water solution, with the dipping filter paper, upon which the adult mosquitoes feed. Photographs were taken at Imperial College's Silwood Park campus.

For the experiment on temperature-dependent survival described here, the mosquitoes were reared in a 12:12 light/dark cycle, at 75% (±5%) relative humidity (RH).

Larvae were placed individually in 12-well plates with 3ml of deionised water two days after hatching, at one of these environmental (air) temperatures: 23 ±1°C, 27 ±1°C, 31 ±1°C, and 35 ±1°C. At every temperature, 640 larvae were reared at a food regime of TetraMin® baby fish food every 24 hours until development into imagoes. On day 2 after hatching from eggs, the larvae were given 0.02 μg of baby fish food per 100 ml of de-ionised water; on days 3, 4, 5, and 6, they were given 0.06, 0.08, 0.16, and 0.32 μg per ml respectively; and from day 7 until pupation, 0.6 μg per ml. The larvae were checked every 24 hours for the emergence of pupae. Pupae were placed in water in a 250ml cup to emerge into adults.

All mosquito immature stages (larvae in the 12-well plates and pupae in the 250ml cups in cages) were checked every 24 hours to monitor survival rates, and the number of dead larvae was recorded at every 24-hour intervals for the construction of life-tables.

As each larva was reared individually, each mosquito is considered an individual data point. The data discussed here is representative only of the mosquito colony used in this experiment, and validation of these results will be ideally required in other mosquito species, and other experimental and field settings.

2.2. Adult maintenance and temperature regimes

Upon emergence from pupae the adults were divided into three groups and placed at either 23°C, 27°C, or 31°C (Figure 2.2). This allowed the distinction between the effects of larval and adult environmental temperatures on adult survival. All mosquitoes reared at 35°C died as larvae, so it was decided not to maintain any adults at 35°C.

Adults were given four days to mate, before the females were placed in individual plastic cups and fed by placing cotton wool soaked in a sugar water solution (10% sugar in distilled water), while the males were discarded.

All dead and live females were counted every 24 hours and the results recorded for the construction of life-tables. All mosquitoes were monitored until 35 days after hatching, when censoring took place and all mosquitoes still alive were frozen.

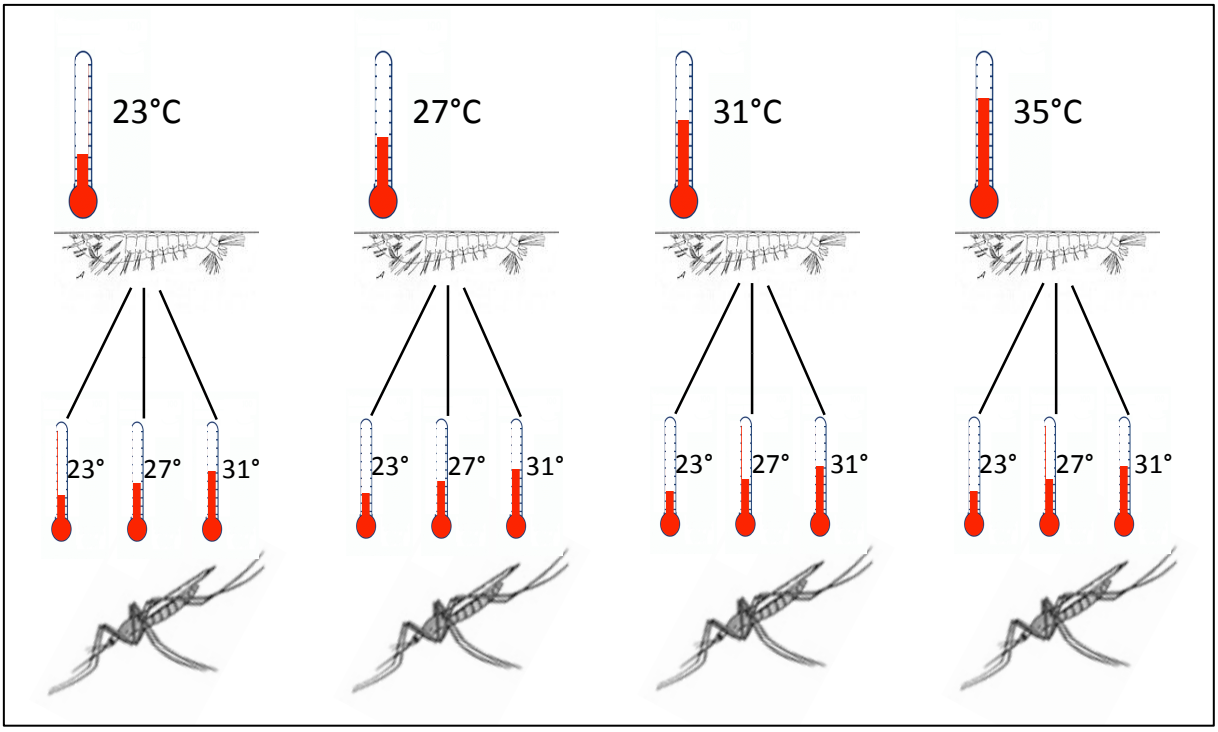


Figure 2.2. Experimental design. Larvae (640) reared at each temperature (23°C, 27°C, 31°C, 35°C) were allowed to develop into imagoes, and the adult females were kept at the same temperature at which they were reared as juveniles, or placed at one of the other temperatures, but not at 35°C: none of the larvae reared at 35°C survived to adulthood, so no adults were maintained at that temperature.



Fig. 2.3. Adult mosquito holding cups. Adult mosquitoes are kept individually in 250ml cups as soon as they emerge from pupae. The cup contains a filter paper dipped in distilled water to keep the inside of the cup humid, and a petri dish is placed in the middle of the cup for the mosquito to rest on. A mesh covers the top of the cup, and cotton wool is placed on top of the mesh and imbibed with sugar water every 48 or 72 hours to provide the mosquitoes with nutrition. Photograph at Silwood Park.

In this chapter only the survival data are presented. Data on larval developmental rates, adult fecundity (number of eggs laid), fertility (number of eggs hatched), and mosquito size (measured by wing length) will be presented in Chapter 3.

2.3. Statistical methods: survival analysis

2.3.1. Non-parametric methods

Survival analyses were performed on each juvenile/adult temperature combination using Kaplan-Meier analysis (Box 1), a standard non-parametric method of representing and analysing survival data (Kaplan & Meier 1958). The Kaplan-Meier analysis was chosen as this is a useful way of comparing median survival times between temperature regimens.

Differences in results from different temperature regimens were compared by the log-rank (Box 2) and Mantel-Cox tests (Box 3), standard methods to test the null hypothesis that survival functions do not differ across groups. The log-rank test was used to compare the overall survival trend for the range of temperatures explored (Collett 2003), and the Mantel-Cox test was used for two-sample comparisons of survivorship at one temperature against the survivorship at the baseline temperature (23°C) (Collett 2003; Mantel & Haenszel 1959). The results are given as test statistics, which when compared with a Chi-squared distribution with one degree of freedom gave the p-values presented in the results section. Mosquitoes frozen on day 35 were classed as censored observations. In order to compare survival times, the median survival time (with 95% confidence intervals) was calculated for each combination of temperature regimes by establishing the time beyond which 50% of the individuals in the population were expected to survive (Collett 2003). This also allowed a useful comparison with datasets from Bayoh and Lindsay (personal communication)These analyses were performed in R, version 3.1.1 (The R Foundation, 2014).

Box 2.1. **The Kaplan-Meier survival function estimate**

The Kaplan-Meier estimate is the nonparametric maximum likelihood estimate of the probability that an individual will live longer than time t, and is given by :

$$\hat{S}(t) = \prod_{j=1}^{k} \left(\frac{n_j - d_j}{n_j} \right)$$

where t_j is the time at which an individual mosquitoes dies, and j = 1, 2, …, r (with r being the total number of "death times" witnessed during the experiment). The intervals between "death times" are described as t_k to t_{k+1}, where k =1, 2, …, r. The number of live individuals at risk of dying before time t_j is given by n_j, and d_j describes the number of mosquito deaths at time t_j (Collett, 2003).

Box 2.2. The log-rank test.

The log-rank test for trend across g ordered groups is based on the following statistic, which has a chi-squared distribution on one degree of freedom (d.f.) when the null hypothesis ("no trend across all g groups") is true (Collett, 2003):

$$W_T = \frac{U_T^2}{V_T}$$

where $$U_T = \sum_{k=1}^{r} w_k \left(\left(\sum_{j=1}^{r_k} d_{kj} \right) - \left(\sum_{j=1}^{r_k} e_{kj} \right) \right) \quad \text{and} \quad V_T = \sum_{k=1}^{g} \left(w_k - \bar{w} \right)^2 e_k$$

In order to test for the effect of temperature on mosquito survival, the groups were defined as the different temperature regimes: 23°C, 27°C, 31°C, and 35°C for larvae; 23°C, 27°C, and 31°C for adults; and the combinations of LT23-AT23 (LT: larval temperature, AT: adult temperature), LT27-AT23, LT31-AT23, LT27-AT23, LT27-AT27, LT27-AT31, LT31-AT23, LT31-AT27, and LT31-AT31.

In the numerator, d_{kj} and e_{kj} describe the observed and expected numbers of deaths in group k (k = 1, 2, …, g) at time t_j. The maximum time mosquitoes survived until they entered group k is given by r_k. w_k is a code assigned to the kth group.

The denominator, V_T, is the variance of U_T, where $\bar{w}$ is a weighted sum of the quantities w_k in which the weights are the expected numbers of deaths, e_k, according to:

$$\bar{w} = \frac{\sum_{k=1}^{g} w_k e_k}{\sum_{k=1}^{g} e_k}$$

Box 2.3. **The Mantel-Cox test.**

The Mantel-Cox test has a chi-squared distribution on one degree of freedom, under the same null hypothesis as the log-rank test, and is based on:

$$W_W = \frac{U_W^2}{V_W},$$

where $$U_W = \sum_{j=1}^{r} \left(d_{1j} - e_{1j} \right) \quad \text{and} \quad V_W = \sum_{j=1}^{r} v_{1j}\,.$$

In the numerator, the number of deaths in the first group at time t_j is given by d_{1j}, and the expected number of deaths in the same group is given by $e_{1j} d_j / n_j$. As in the Kaplan-Meier funciton, n_j is the number of mosquitoes at risk of dying before time t_j.

The denominator, V_W, is the variance of U_W, given by:

$$v_{1j} = \frac{n_{1j} n_{2j} d_j \left(n_j - d_j \right)}{n_j^2 \left(n_j - 1 \right)},$$

where 1 and 2 are the two temperature groups we are comparing (Collett, 2003).

2.3.2. Parametric methods

To test the widely used assumption that the survival of adult *Anopheles* follows a model of time-independent mortality, the method of maximum likelihood estimation (MLE) was used. This method was chosen over the Least Squares Estimation (LSE) method as the estimated variance is smaller when using MLE, and the estimates of the distribution parameter are more precise. In addition, it has been shown that in the particular case of the Weibull functional form, the LSE results can be misleading and suggest a fit where the Weibull form is actually inappropriate (Genschel and Meeker 2010).

MLE was therefore used to fit the survival functions of four functional forms (exponential, gamma, Gompertz, and Weibull (Collett 2003)) to larval and adult survival data at each temperature regime, and the hazard function was calculated for the best-fitting form. These particular functional forms (gamma, Gompertz, and Weibull) were chosen as they are all used to model age-dependent survival in medical research, and therefore were all expected to provide viable alternatives to the exponential form (which implies a constant, age-independent survival) which is most commonly used in modeling vector survival.

The exponential model (equation 2.1) implies a constant mortality rate,

$$f(t) = \lambda e^{-\lambda t}, \tag{2.1}$$

while the gamma (equation 2.2), Gompertz (equation 2.3), and Weibull (equation 2.4) models all allow for age- (or time-) dependent mortality:

$$f(t) = \frac{\beta^{\alpha} t^{\alpha-1} e^{-\beta t}}{\Gamma(\alpha)}, \tag{2.2}$$

where $\Gamma(\alpha)$ is the gamma function evaluated at α;

$$f(t) = \lambda e^{\theta t} \exp\left\{\frac{\lambda}{\theta}\left(1 - e^{\theta t}\right)\right\} \tag{2.3}$$

$$f(t) = \lambda \gamma t^{\gamma-1} \exp\left(-\lambda t^{\gamma}\right). \tag{2.4}$$

The log-likelihood function (log-L) was used to fit the survivor functions of the function forms described above to the survival data,

$$\log L = \sum_{t} N_t S(t) + \left(N_o - N_t\right)\left(1 - S(t)\right) \tag{2.5}$$

where N_0 is the number of larvae or adults alive at the start of the experiment, $N(t)$ is the number alive at the beginning of day t and $S(t)$ is the probability of surviving to day t according to the fitted survivorship function. The parameter values of the best-fit functional

form at each temperature regime were input into the related hazard function to describe mortality at all temperature regimes, and the uncertainty around the parameters obtained by MLE at each temperature regime was calculated with the profile likelihood method (McCallum 2000).

The goodness-of-fit of the different models was compared by Akaike Information Criterion (AIC) (equation 2.6), or by AICc (corrected Akaike Information Criterion) (equation 2.7) when the sample size was smaller than 80 to avoid over-fitting (Bolker 2008; Burnham & Anderson 2002):

$$AIC = -2(\log L) + 2k \tag{2.6}$$

$$AICc = AIC + \frac{2k(k+1)}{n-k-1} \tag{2.7}$$

where k is the number of parameters in the model, and n is the sample size.

A difference of ≤2 in AIC values suggests that two fits are not significantly different, and only models with a difference of >4 in AIC values are statistically dissimilar (Bolker 2008).

These analyses were performed in Microsoft Excel for Mac 2011, version 14.5.9.

3. Results

3.1. The effect of larval environmental temperature on larval survival

It was only possible to estimate the median survival time (8 days) for larvae reared at 35°C (Table 2.4) as for the lower temperature regimes the survivorship curves did not cross 0.5 (Figure 2.5A). The Kaplan-Meier plots show that larval mortality increased substantially with increasing environmental temperature (Figure 2.5A). The overall trend reported a statistically significant increase in mortality as temperatures increased ($p<0.001$) (Table 2.5). The increase in larval mortality was significant with the 4°C increase in temperature from 23°C to 27°C ($p<0.001$), as well as with the 8°C increase from 27°C to 35°C ($p<0.001$), and the 12°C increase from 23°C to 35°C ($p<0.001$). The results of an 8°C increase in temperature from 23°C to 31°C did not allow for a useful statistical test.

Increases in larvae mortality were also significant when the temperature increased by 4°C above temperatures other than the baseline (23°C): increases from 27°C to 31°C and from 31°C to 35°C both resulted in significant decreases in larvae survival with $p<0.001$. All larvae kept at 35°C died before they could emerge into adults.

Table 2.1. **Median survival times of *An. gambiae* s.s. larvae at different environmental temperatures.** *ND: Not determined. Median survival defines the time point at which the survivorship curve crosses 0.5, or at which 50% of the sample is expected to survive. In this case, the survival function did not cross 0.5, and the median survival cannot be calculated.

Temperature (°C)	Total number of larvae exposed	Median survival (days) (95% C.I.)
23±1	639	ND*
27±1	589	ND*
31±1	638	ND* (9, ND)
35±1	636	8 (8, 8)

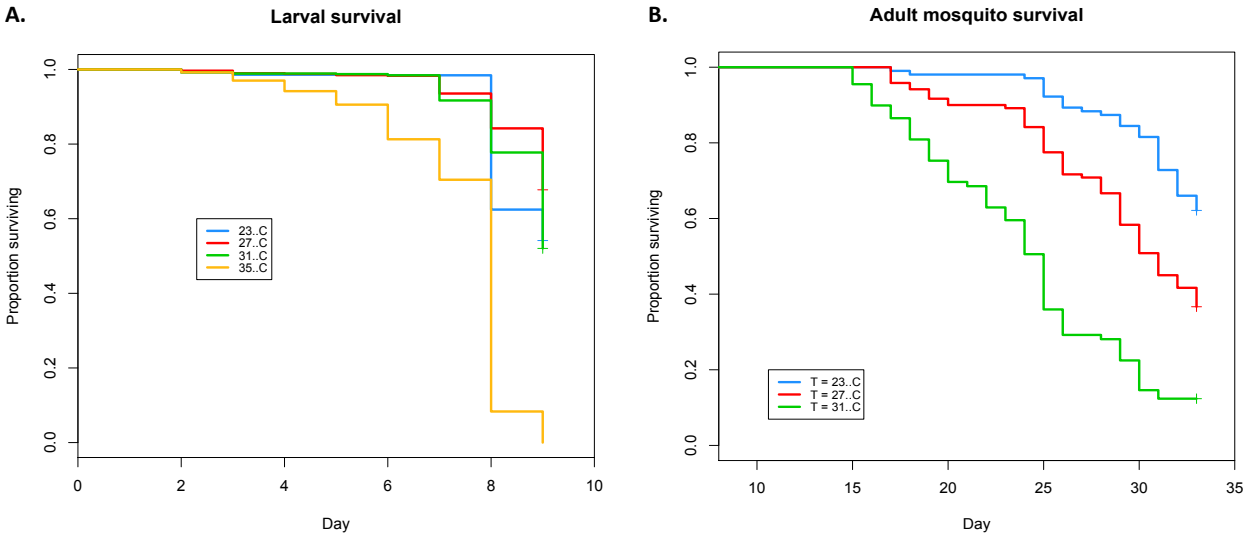


Figure 2.4. **Kaplan-Meier plots.** *An. gambiae* larval (A) and adult (B) survival at different environmental temperatures. 23°C (red in A, blue in B) was set as the baseline against which survival at other temperature was compared; 27°C (blue in A, red in B); 31°C (green in A and B); 35°C (yellow in A, no adults were kept at 35°C).

Table 2.2. The effect of larval temperature on larval survival. Two-group comparisons and overall trend of the effect of larval environmental temperature on larval survival. The comparison between 31°C and 23°C showed partly indistinguishable data, and did not allow for meaningful statistical tests.

Test statistic	27±1°C (wrt 23°C)	31±1°C (wrt 27°C)	35±1°C (wrt 23°C)	35±1°C (wrt 27°C)	35±1°C (wrt 31°C)	Overall effect of temperature on larval survival	
Mantel-Cox test	28.97	29.63	642.10	932.70	798.60	Log-rank test	1510.00
p-value	<0.001	<0.001	<0.001	<0.001	<0.001	p-value	<0.001

3.2. The effect of adult environmental temperature on adult survival

Table 2.6 and Figure 2.5B show that, while the survivorship curve did not cross 0.5 for adult female mosquitoes kept at 23°C and it is therefore not possible to compute the median survival time at this temperature, it was possible to ascertain that median survival decreased from 31 days at 27°C to 25 days at 31°C. Overall, higher temperatures resulted in an increase in adult mortality ($p<0.001$) (Table 2.7 and Figure 2.5B). Adult mortality was significantly more elevated with every increase in temperature relative to the baseline of 23°C: p-values were all highly significant ($p<0.001$) for comparisons of 27°C vs. 23°C and 31°C vs. 27°C (4°C increase in both cases), and 31°C vs. 23°C (8°C increase, Table 2.7).

Table 2.3. Median survival times of *An. gambiae* adults at different environmental temperatures.

Temperature (°C)	Total number of larvae surviving to imagoes	Median survival (days) (95% C.I.)
23±1	103	ND*
27±1	120	31 (30, 33)
31±1	89	25 (24, 25)

Table 2.4. Two-group comparisons and overall trend of the effect of adult environmental temperature on adult survival.

Test statistic	27°C±1 (with respect to 23°C)	31°C±1 (with respect to 23°C)	31°C±1 (with respect to 27°C)	Overall effect of temperature on adult survival	
Mantel-Cox test	17.95	92.40	37.23	Log-rank test	102.30
p-value	<0.001	<0.001	<0.001	p-value	<0.001

3.3. The effect of larval environmental temperature on adult survival

Table 2.5 outlines the median survival times for each combination of adult temperatures and temperatures at which these adults had been reared as immature stages. There is a trend showing decreasing median survival times of adult females with increasing adult temperature. Within each group, median survival times tend to be higher when larvae and adults are maintained at similar environmental temperatures than when their temperatures are more divergent; for example, when both the larvae and the adults are exposed to 31°C, median survival time is 26 days, but only 22 days for adults that had been reared at 23°C as larvae.

Table 2.5. Median survival times of adult *An. gambiae*. Survival times depend on the temperature of the adult environment, and the temperature at which the larvae that developed into such adults had been reared.

Adult temperature (°C)	Larval temperature (°C)	Total number of adults exposed at the start	Median adult survival (days) (95% C.I.)
23±1	23±1	39	ND*
	27±1	40	ND*
	31±1	24	32.0 (30, ND)
27±1	23±1	40	33.0 (31, ND)
	27±1	40	33.0 (31, ND)
	31±1	40	28.5 (25, 30)
31±1	23±1	26	22.0 (19, 25)
	27±1	40	25.0 (22, 28)
	31±1	23	26.0 (25, 30)

The temperature at which the larval stages were reared was found to have a significant effect on the survival of adult mosquitoes. When larvae reared at different temperatures were placed at 23°C as adults, those that had been reared at 27°C did not experience significantly higher mortality than those reared at 23°C ($p=0.92$). However, mosquitoes that were reared at 31°C had a higher mortality than those that were reared at 27°C ($p<0.05$), and those that had been exposed to an 8°C decrease (from 31°C as larvae to 23°C as adults) suffered a statistically significant increase in mortality compared to adults reared at 23°C ($p<0.05$) (Figure 2.5A and Table 2.6).

For larvae reared at all temperatures (23, 27, 31°C) and moved to 27°C as adults, there was no significant difference in adult survival between those reared at 27°C and those reared at 23°C ($p=0.927$), while mosquitoes exposed to a 4°C decrease in temperature between the larval and the adult stages (from 31°C as juveniles to 27°C as adults) experienced a significantly higher adult mortality ($p<0.001$) (Figure 2.5B and Table 2.6).

Finally, for adults kept at 31°C, mosquitoes experiencing a 4°C increase in temperature (from 27°C as larvae to 31°C as adults) experienced no difference in survival ($p=0.182$), but an 8°C increase (from 23°C as larvae to 31°C as adults) significantly decreased adult survival ($p<0.01$). The effect of larval temperature on adult survival was significant no matter the temperature at which the adults were kept (23°C ($p<0.05$), 27°C ($p<0.001$), and 31°C ($p<0.05$)) (Figure 2.5), and larval environmental temperature significantly affected adult survival, at all adult temperatures ($p<0.001$) (Table 2.9).

Table 2.6. The effect of larval temperature on adult survival. Two-group comparisons and overall trend of the effect of larval environmental temperature on the survival of adult mosquitoes, at different adult environmental temperatures.

		Larval temperature (°C)			Overall effect of larval temperature on adult survival	
Adult temperature (°C)	**Test statistic**	**27±1 (with respect to 23°C)**	**31±1 (with respect to 23°C)**	**31±1 (with respect to 27°C)**	**Test statistic**	
23±1	Mantel-Cox test	0.01	4.88	4.63	Log-rank test	6.51
	p-value	0.920	0.027	0.031	p-value	0.039
27±1	Mantel-Cox test	0.01	16.29	19.43	Log-rank test	23.51
	p-value	0.927	<0.001	<0.001	p-value	<0.001
31±1	Mantel-Cox test	2.74	7.41	1.78	Log-rank test	7.61
	p-value	0.098	0.006	0.182	p-value	0.022
All adult temperatures					Log-rank test	108.30
					p-value	<0.001

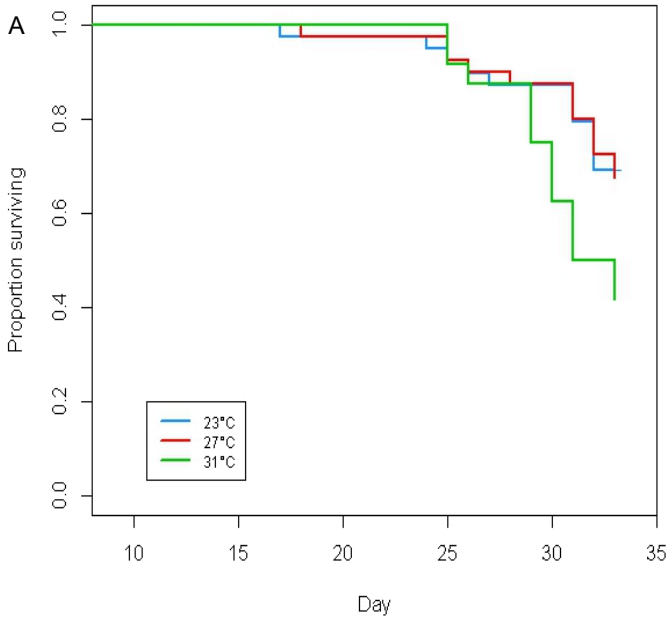
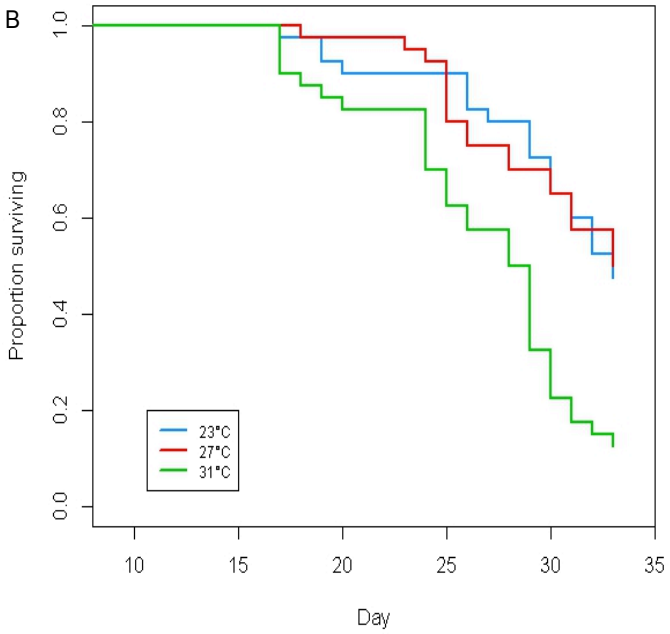


Figure 2.5. Kaplan-Meier plots of adults reared at different larval temperatures. Survival of adults kept at different environmental temperatures having been reared as larvae at different temperatures. **A**, Adult survival curves at adult environmental temperature of 23°C. Larval temperature 23°C (blue) was set as the baseline against which survival at other larval temperatures was compared; 27°C (red); 31°C (green).



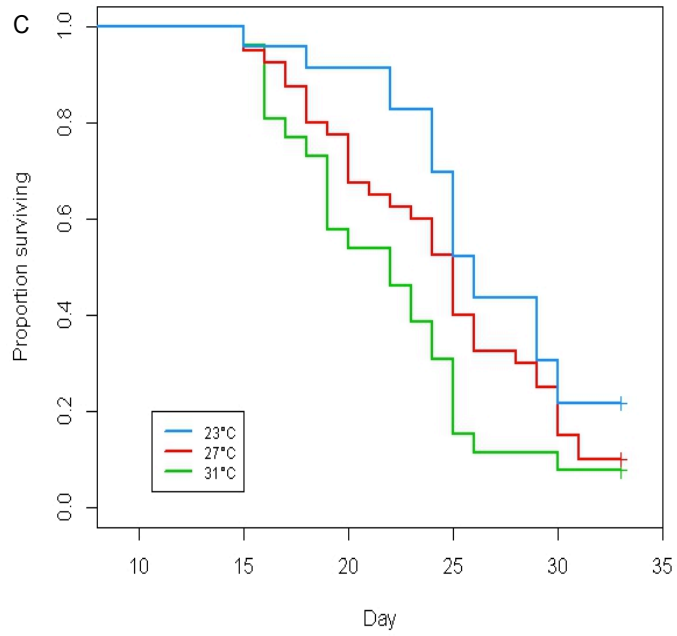


Figure 2.5. B. Adult survival curves at adult environmental temperature 27°C. As in **A**, larval temperature 23°C (blue) was set as the baseline against which survival at other larval temperatures was compared; 27°C (red); 31°C (green).
Figure 2.5. C. Adult survival curves at adult environmental temperature 31°C. Larval temperatures as in **A** and **B**.

3.4. Parametric curve fitting to survival data

The Gompertz functional form fit the survival data better than the exponential, gamma and Weibull survival functions in 10 out of 16 temperature scenarios, and was not significantly worse than the best fit in 2 further cases (Tables 2.7-2.9, Figure 2.6). Figures 2.7 and 2.8 show the best-fit Gompertz survival curves for all combinations of larval and adult temperatures. The implications for modelling *Anopheles* population dynamics and malaria transmission are that an age-dependent mortality model is likely more appropriate than a model assuming constant mortality.

Table 2.7. Akaike Information Criterion (AIC) values. Values given for the exponential, gamma, Gompertz, and Weibull fits to larval survival data (* indicates the best fit).

Larval temperature	Parametric curve	AIC value
23°C	exponential	3075.58
	gamma	2640.55
	Gompertz	2473.30 *
	Weibull	2560.13
27°C	exponential	2202.65
	gamma	1990.12
	Gompertz	1923.87 *
	Weibull	1964.27
31°C	exponential	2926.08
	gamma	2531.45
	Gompertz	2374.34 *
	Weibull	2459.54
35°C	exponential	4725.81
	gamma	3360.68
	Gompertz	2835.85 *
	Weibull	3047.92

Table 2.8. Akaike Information Criterion (AIC) values. Values given for the exponential, gamma, Gompertz, and Weibull fits to adult survival data (* indicates the best fit).

Adult temperature	Parametric curve	AIC value
23°C	exponential	1123.14
	gamma	1034.29
	Gompertz	1023.79 *
	Weibull	1029.24
27°C	exponential	2134.52
	gamma	1998.65
	Gompertz	1990.06 *
	Weibull	1992.33
31°C	exponential	1966.31
	gamma	1928.30 *
	Gompertz	1934.92
	Weibull	1928.94

Table 2.9. AICc values. Values given for the exponential, gamma, Gompertz, and Weibull fits to adult survival data, subdivided by larval temperature (* indicates the best fit, ‡ indicates where the Gompertz fit is not significantly worse than the best fit).

Adult temperature	Larval temperature	Parametric function	AICc value
23°C	**23°C**	exponential	410.32
		gamma	395.2
		Gompertz	392.28 *
		Weibull	394.24
	27°C	exponential	398.36
		gamma	378.09
		Gompertz	375.73 *
		Weibull	377.12
	31°C	exponential	308.01
		gamma	234.92 *
		Gompertz	239.62
		Weibull	236.42
27°C	**23°C**	exponential	619.1
		gamma	592.19
		Gompertz	588.35 *
		Weibull	590.66
	27°C	exponential	594.63
		gamma	539.22 *
		Gompertz	544.83
		Weibull	539.76
	31°C	exponential	802.8
		gamma	730.66
		Gompertz	717.53 *
		Weibull	723.72
31°C	**23°C**	exponential	756.57
		gamma	752.92 *
		Gompertz	758.79
		Weibull	754.35
	27°C	exponential	831.16
		gamma	787.32
		Gompertz	785.38 ‡
		Weibull	784.87 *
	31°C	exponential	460.54
		gamma	418.8
		Gompertz	416.28 ‡
		Weibull	415.49 *

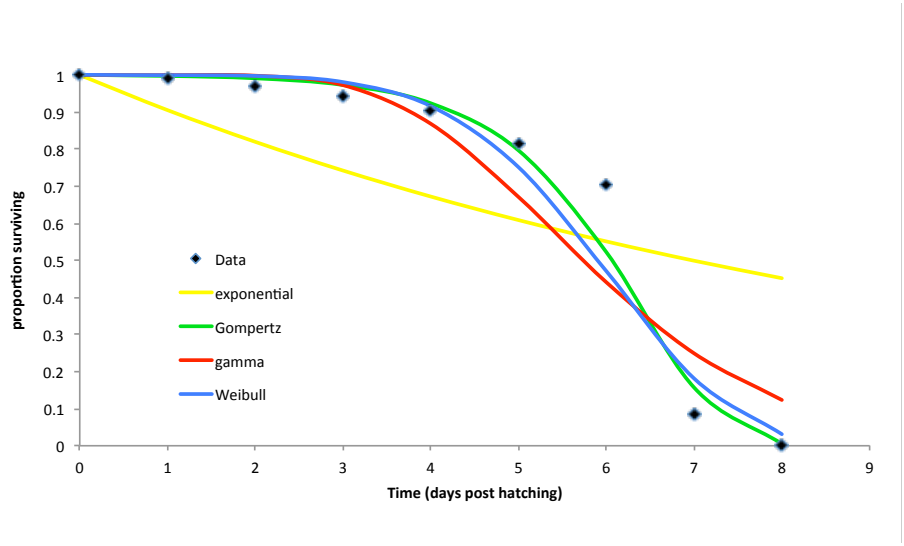


Figure 2.6. Parametric fitting. An example of the fitting of four parametric survival functions (exponential (yellow), Gompertz (green), gamma (red), and Weibull (blue)) to larval survival data at environmental temperature 35°C.

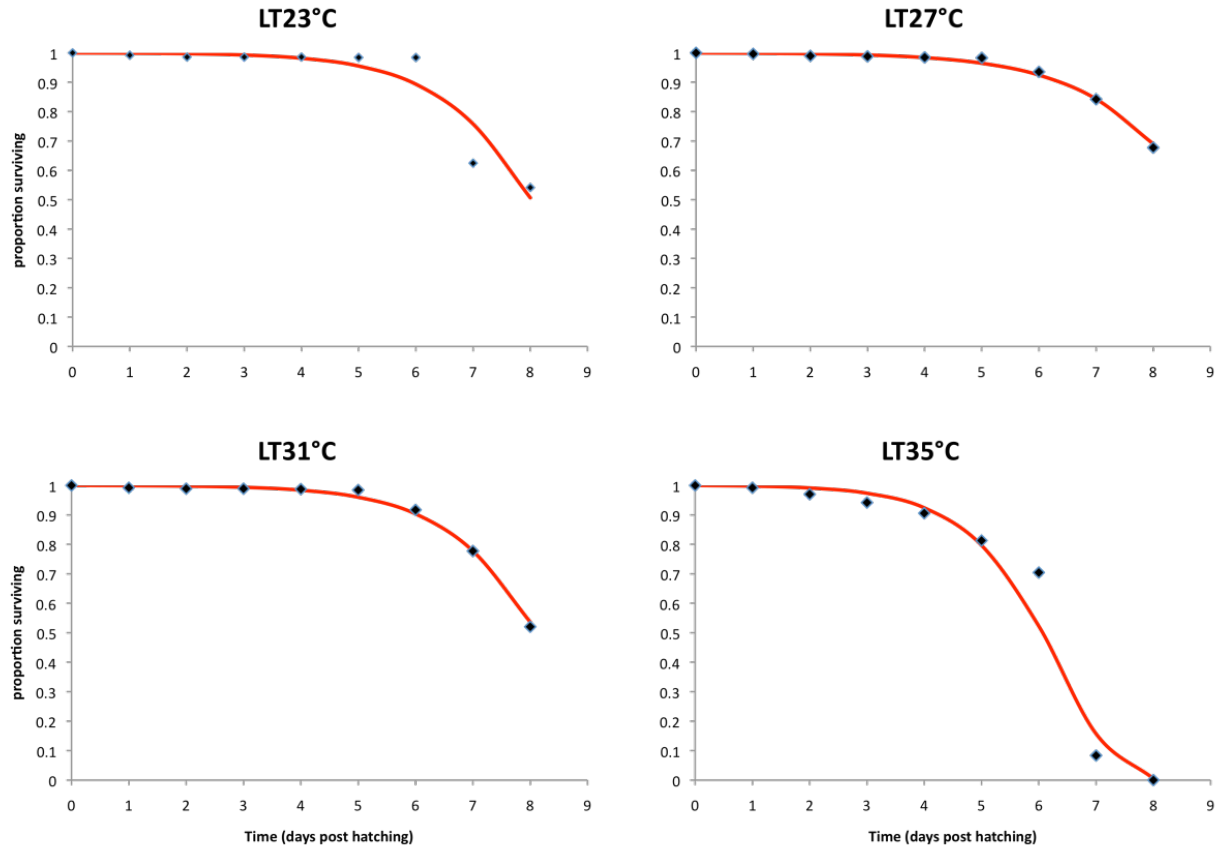


Figure 2.7. Gompertz fits to larval survival data. The Gompertz survival functions (red) are shown alongside the larval survival data at all environmental temperatures (23°C, 27°C, 31°C, and 35°C) to which they were fitted.

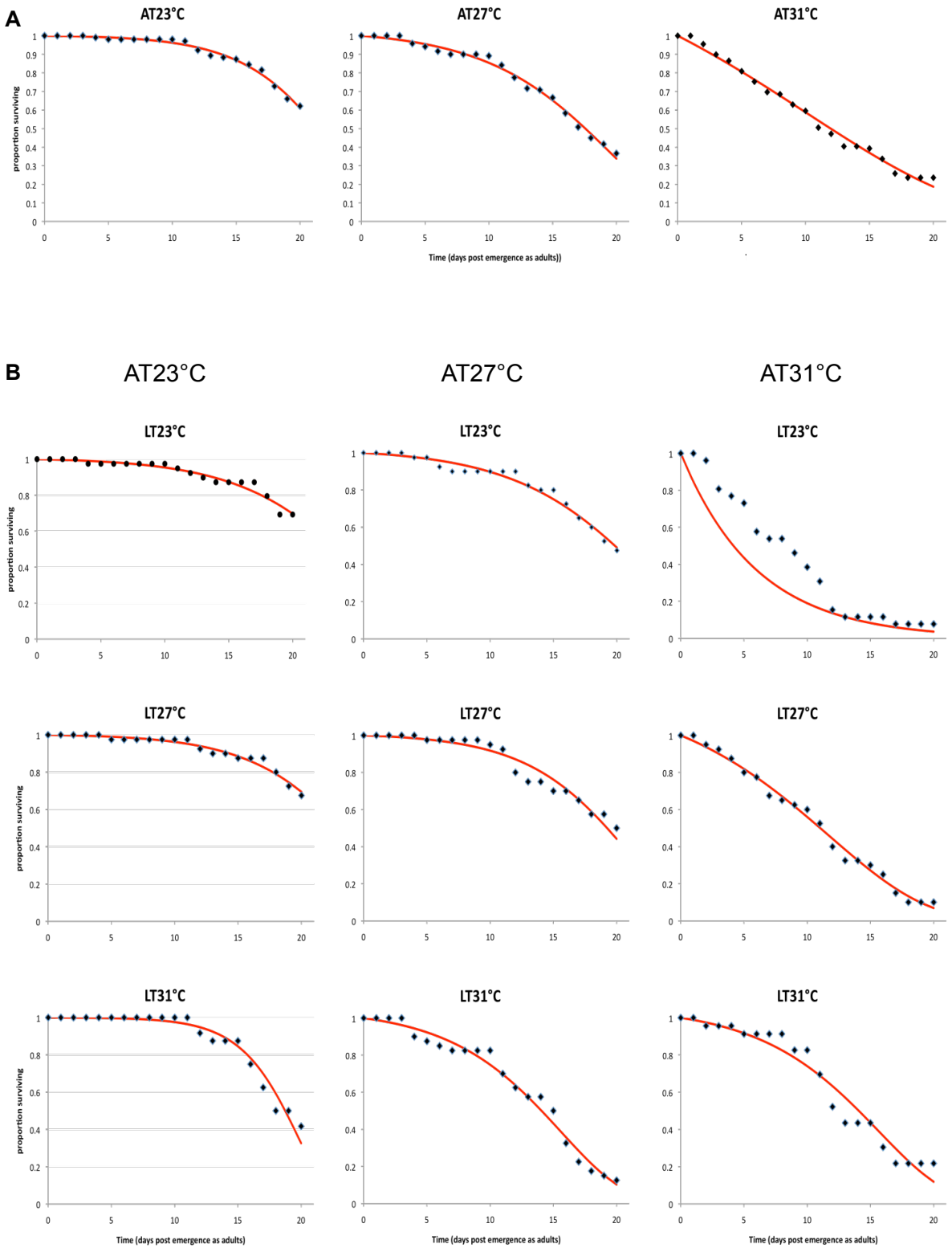


Figure 2.8. Gompertz fits to adult survival data. A, The Gompertz survival functions (red) are shown alongside the adult survival data at all adult temperatures (23°C, 27°C, 31°C) to which they were fitted. B, The Gompertz survival functions (red) are shown alongside the adult survival data at all combinations of larval and adult temperatures to which they were fitted.

The parameter values defined by MLE as yielding the best-fit function were used to develop Gompertz hazard functions,

$$h(t) = \lambda \exp(\theta t) \exp\left\{\frac{\lambda}{\theta}(1 - \exp(\theta t))\right\}, \quad (2.8)$$

which were plotted against the mortality data for each temperature regimen (Fig. 2.9 and 2.10).

Figure 2.11 shows the values for the two parameters of the Gompertz survival function, λ and θ, and their 95% confidence intervals (CI) at each temperature. Figures 2.11A and 2.11B show that both parameters of the Gompertz survival functions vary widely as a function of adult temperature, whereas adult temperature induces significant changes in λ only. Figure 2.11C shows that for the Gompertz curves describing adult survival at adult temperatures of 23°C and 27°C, both λ and θ remain relatively unaffected by the temperature at which the larvae were reared, but change with larval temperature when adults were maintained at 31°C.

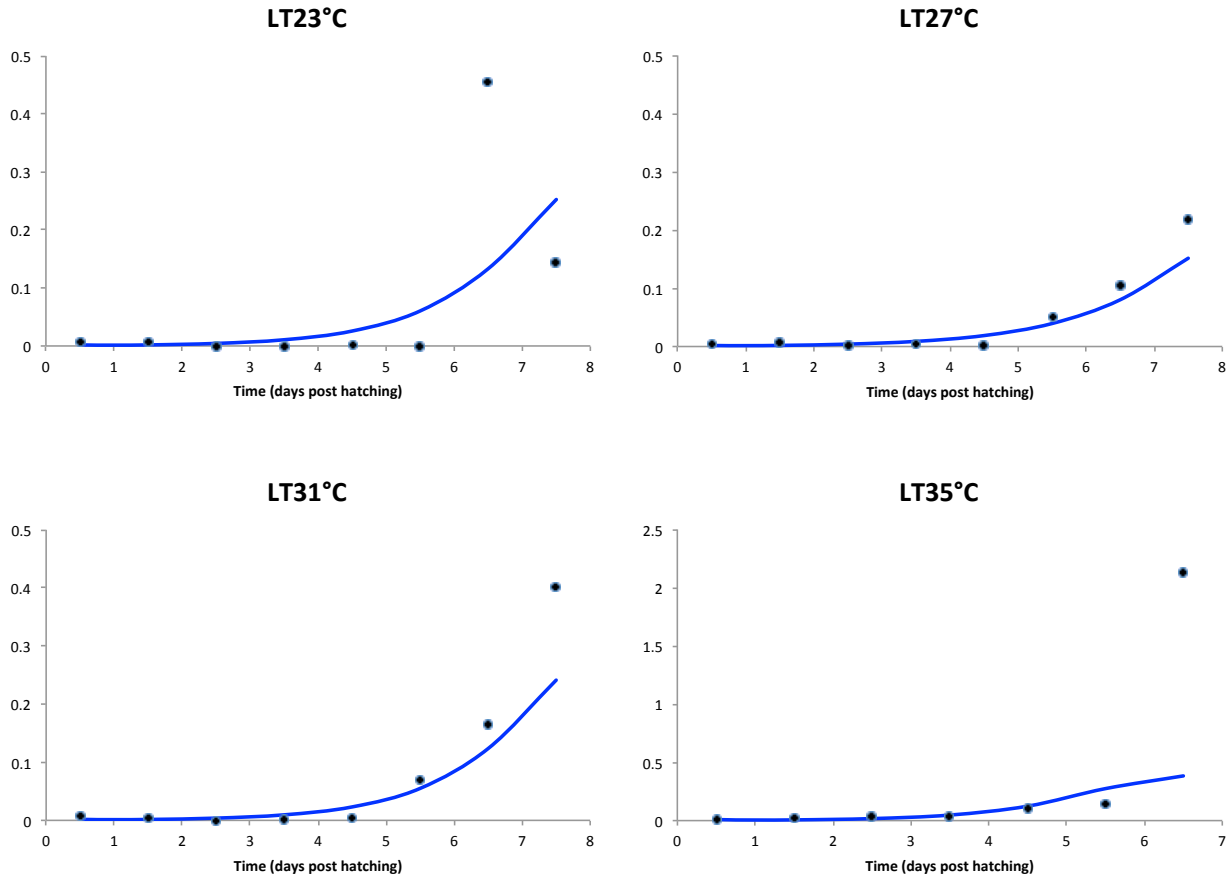


Figure 2.9. Best-fit Gompertz hazard function plotted against larval survival data. The Gompertz functions (blue) are shown alongside the larval survival data at all environmental temperatures (23°C, 27°C, 31°C, and 35°C).

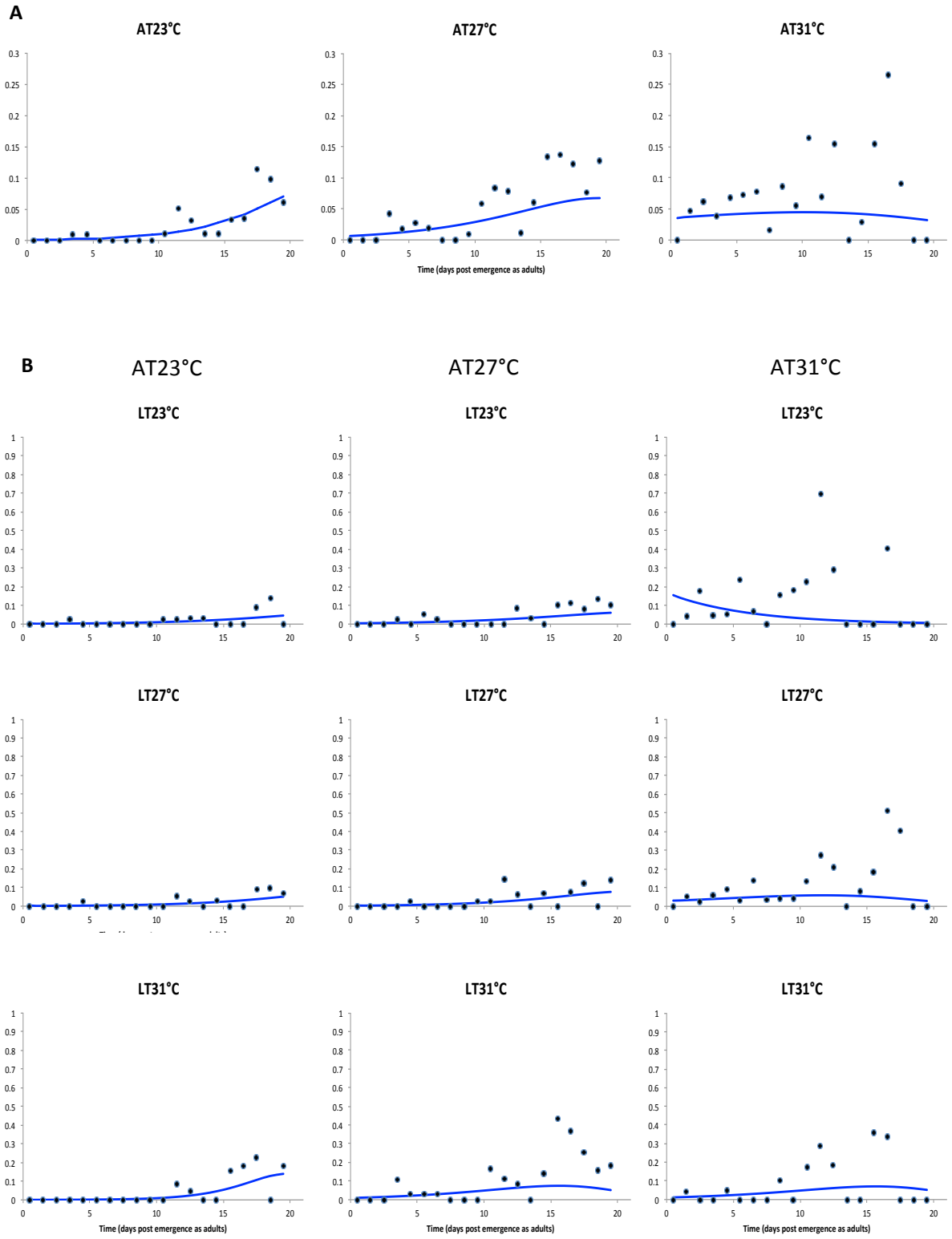


Figure 2.10. Gompertz hazard function plotted alongside adult mortality data. A, Gompertz functions (blue) are shown alongside the adult mortality data at all adult temperatures (23°C, 27°C, 31°C). B, Gompertz functions (blue) are shown alongside the adult mortality data at all combinations of larval and adult temperatures.

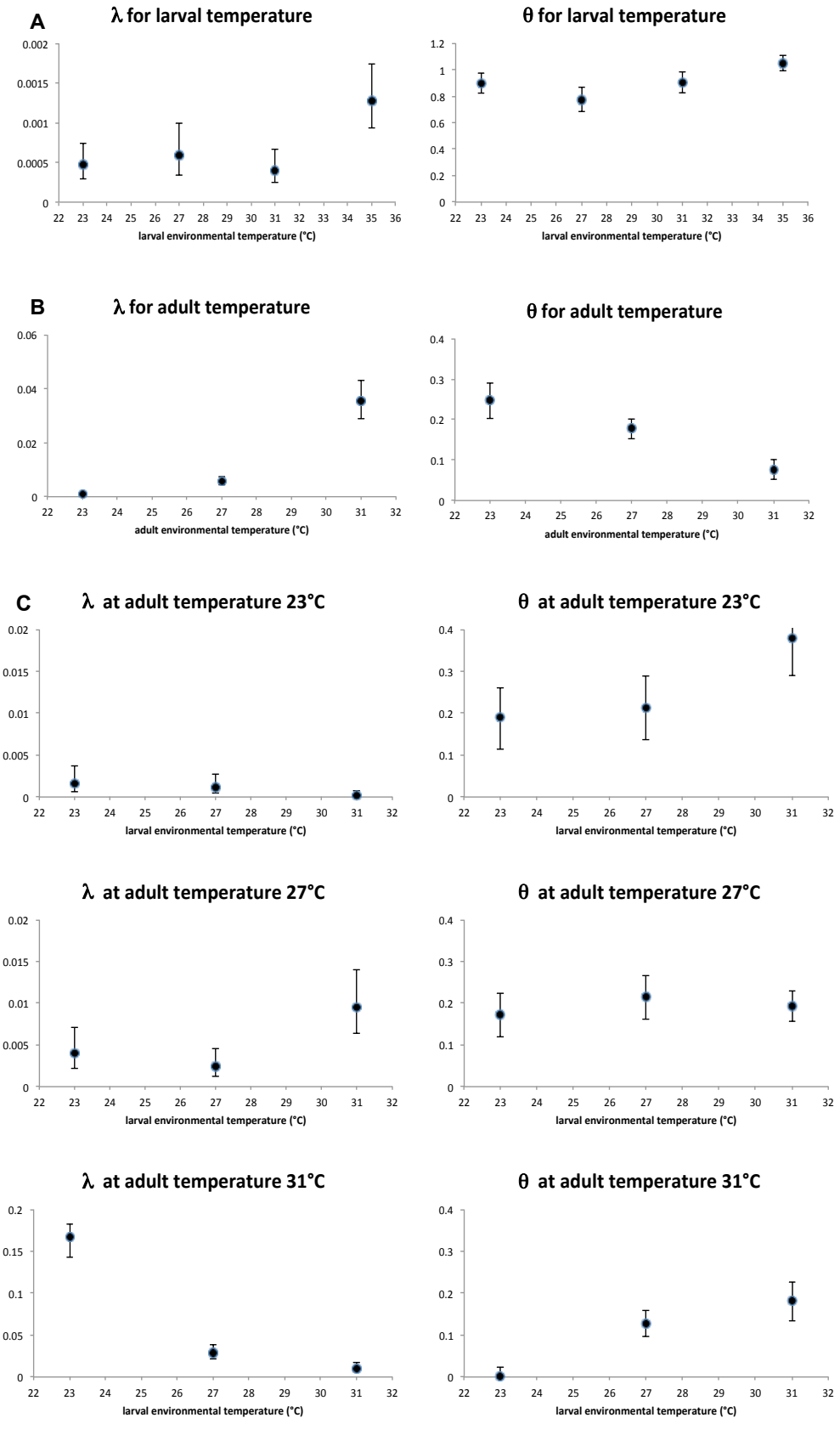


Figure 2.11. Values of the Gompertz survival function parameters, λ and θ. A, Parameters for the Gompertz survival function fitted to the larval survival data at each larval temperature are shown with their 95% confidence intervals (CI). B, Parameters for the Gompertz survival function fitted to the adult survival data at each adult temperature are shown with their 95% CI. C, Parameters for the Gompertz survival function fitted to the adult survival data at each combination of larval and adult temperatures are shown with their 95% CI.

4. Discussion

The results discussed here demonstrate that environmental temperature affects the survival of *Anopheles gambiae* s.s., during all stages of the mosquito's development and during their lifetime as adults. Results of the larval survival experiments showed a significant increase in larval mortality with every 4°C increase in air temperature, which agrees with previous studies (Bayoh & Lindsay 2004; Bayoh & Lindsay 2003; Depinay et al. 2004). There was also a significant increase in adult mortality with each 4°C increase in environmental temperature, as has also been described in other research (Armstrong & Bransby-Williams 1961; Kirby & Lindsay 2009; Lunde et al. 2013).

However, this is the first study to investigate the effect of the environmental temperature during larval development on adult *Anopheles gambiae* survival. The results reveal that a small difference (4°C) in the temperatures of the larval and adult environments may have a significant impact on adult survival, and that the magnitude of this impact may depend on the respective temperatures. This suggests that the temperature of the larval environment may influence the adult stages much more than previously thought, and that the adult mosquito may be extremely sensitive to the temperatures and combinations of temperatures to which it is exposed during its lifetime.

As a result of the complexities of the experimental setup and the logistical constraints limiting the number of mosquitoes that could be reared and observed, only four temperature regimes and only temperature increases of 4°C were investigated for this thesis, which makes it difficult to extrapolate these conclusions to smaller increases in temperature. In addition, this experimental setup exposed the mosquitoes to one fixed temperature, and did not allow for diurnal and nocturnal temperature fluctuations, or for any temperature fluctuations whatsoever. This is not representative of the mosquito's environment in the wild, and may therefore have biased the results.

The Gompertz survivorship function fitted the survival data well generally, which is consistent with the results of Clements and Paterson (Clements & Paterson 1981a) and suggests that age-dependent mortality (senescence) is a relevant factor both in the immature and adult stages of the mosquito's life, at least in laboratory conditions. Dawes *et al*. also documented age-dependent mortality in adult populations of *An. stephensi* in the laboratory (Dawes et al. 2009). Senescence has been reported in *Aedes aegypti*, both in laboratory and in semi-field conditions (Styer, Minnick, et al. 2007; Harrington et al. 2008). As indicated by other authors (Clements & Paterson 1981a; Styer, Minnick, et al. 2007; Hancock et al. 2009;

Brady et al. 2013; Parham et al. 2012), VBD models assume there is insufficient evidence supporting age-dependent vector mortality (Yang et al. 2009) for the sake of tractability, and because there exists contradictory evidence between laboratory and field studies (Dawes et al. 2009; Bellan 2010; Ferguson & Read 2002). VBD models therefore often assume a constant hazard (and thereby an exponential distribution of mortality, shown here to fit poorly to the data) (Wilson 1994). In addition, the data presented here indicate that age-dependent mortality may depend on environmental temperature both in the immature and in the adult stages of *Anopheles gambiae* s.s. mosquitoes. This may have significant consequences for predictions of mosquito abundance, as up until now senescence has not been taken into account: the evidence of temperature-dependent senescence described here suggests this may actually play an important role in the mosquito's life history, its reproduction, and its ability to stay alive long enough, at different temperatures, to transmit malaria.

These results give a thorough representation of larval survival at a range of temperatures. Previous research has investigated the effect of temperature on mortality rates in larvae (Bayoh & Lindsay 2004), the proportion of larvae surviving to adulthood (Bayoh & Lindsay 2003; Kirby & Lindsay 2009), the combined influence of larval density and environmental temperature on larvae survival rates (Lyimo et al. 1992), the combined influence of inter-species competition and environmental temperature on the percentage of larvae developing to adults (Kirby & Lindsay 2009), and the influence of altitudinal changes in temperature on the percentage of larvae that develop to adults (Afrane et al. 2007b). This experiment is, to my knowledge, the first to follow a generation of *Anopheles gambiae* s.s. larvae during their entire lifecycle.

Previous research into adult mortality has investigated daily survival probabilities within a range of temperatures from 5°C to 40°C and with a humidity range from 40% to 100% (Midega et al. 2007; Olayemi & Ande 2008; Bayoh & Lindsay (pers.comm.)), the different mortalities of males and females when emerging into adults (Armstrong & Bransby-Williams 1961), the time to 50% survival at different temperatures (Lardeux et al. 2008), the proportion surviving after exposure to high temperatures (Kirby & Lindsay 2004), and survival at different combinations of temperature and relative humidity (RH) (Boyd 1949; Bayoh & Lindsay (pers.comm.)). However, this study differs from previous research in that mosquitoes were allowed to blood-feed and oviposit, simulating more accurately their true life cycle.

An additional laboratory experiment was undertaken to allow for replicates and to provide further data, but this did not complete the experimental protocol as most mosquitoes died

whilst in their larval stages, and those that made it to adulthood did not survive long. It was conjectured there may have been an infection in the mosquito colony, as most larvae did not feed.

The results on adult mortality at a larval temperature of 27°C and adult temperatures of 23°C, 27°C, and 31°C, with 75 ± 5% RH, were informally compared to those reported by Bayoh and Lindsay (Bayoh & Lindsay (pers.comm.)) at 20°C, 25°C, and 30°C, with 80% RH, and found similar survival curves (Figure 2.12). The greater mortality in this study is probably due to differences in our experimental protocols. Host-seeking, blood feeding, and oviposition all carry fitness and survival costs, in that metabolic vector resources are allocated to reproduction, and that the mosquito incurs a risk of drowning while laying, and in placing additional stress associated with displacement on the mosquito (Lyimo & Ferguson 2009; Ferguson 2012). Differences between laboratory studies in malaria vector survival may be, partly, associated with allowing or not blood meals and oviposition (Heather Ferguson, pers. comm.).

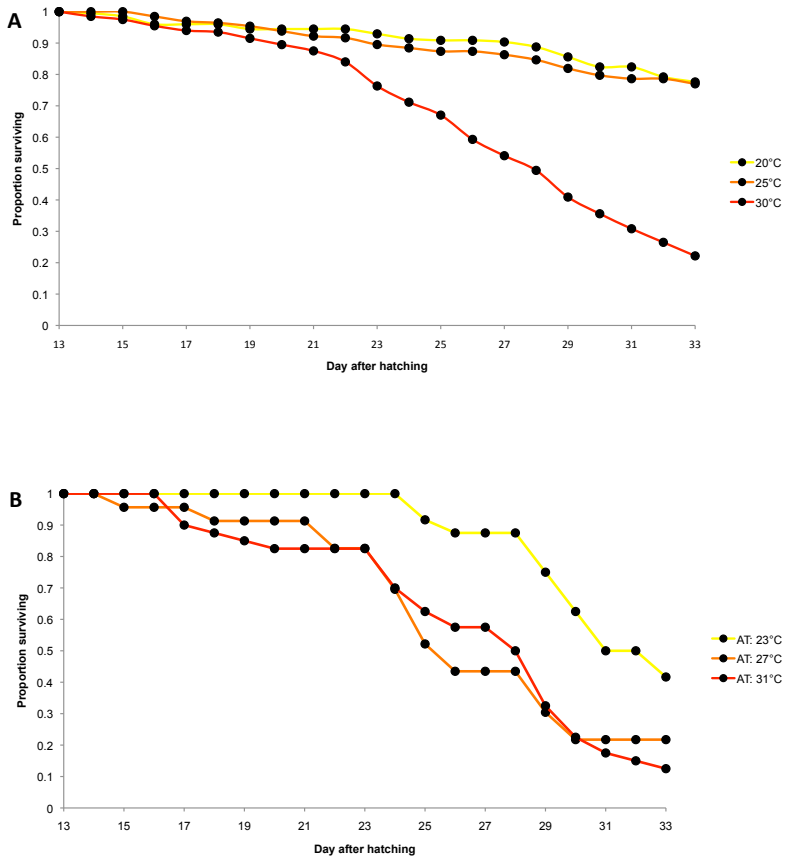


Figure 2.12. A comparison of survival curves. **A**, Survival curves by Bayoh and Lindsay (pers.comm.), larval temperature 26°C, 80% RH. Adult survival at environmental temperature 20°C (yellow), 25°C (orange), and 30°C (red). **B**, Survival curves with data from this study, larval temperature 27°C, 75% RH. Adult survival at environmental temperature 23°C (yellow), 27°C (orange), 31°C (red).

It appears that the extent to which *An. gambiae* larval temperature influences adult survival depends on the environmental temperature of the adults. Further experiments are needed to determine whether an increase of a certain magnitude in larval temperature will only affect adult survival within certain adult temperature margins or whether a threshold exists above which increasing larval temperature significantly reduces adult survival.

The design of this study did not take into consideration temperature or humidity fluctuations that might affect mosquito development and survival in the field, nor the air-water temperature difference: only the air temperature was measured. Further research is required to investigate how other climatic and environmental factors may affect *An. gambiae* survival and development rates. Further investigation is also needed into the influence of localised air temperature fluctuations and how these influence the water temperature of mosquito breeding sites. Finally, *Anopheles gambiae* s.s. is merely one of the seven dominant vector species of malaria in humans on the African continent (Sinka et al. 2012), and data on the sensitivity of other species to temperature and other climate- and population-related factors are equally scarce, if not more so. Climate change and global warming are likely to affect different species of malaria vectors' survival (Lyons et al. 2013) and life-history parameters (Sinka et al. 2010) in different manners. More comprehensive and species-specific data on the sensitivity of mosquito population dynamics and life-history parameters on climatic factors, coupled with geographically detailed climate forecasts, will allow more reliable and robust predictions of vector population dynamics, and thereby of disease transmission.

This chapter presents evidence for the first time that *An. gambiae* s.s. survival is both temperature- and age-dependent, and that the environmental temperature in which the mosquito's immature stages develop influence adult survival. This leads to the expectation that the environmental temperature of the larvae may also affect other life-history parameters. The following chapter examines the impact of temperature throughout the life of *An. gambiae* s.s. mosquitoes on several life-history relevant to population dynamics.

CHAPTER 3

THE EFFECT OF ENVIRONMENTAL TEMPERATURE DURING LARVAL AND ADULT STAGES ON *ANOPHELES GAMBIAE* S.S. LIFE-HISTORY AND REPRODUCTIVE PARAMETERS

1. Introduction

As discussed in the previous chapter, climatic factors are expected to affect the geography and transmission of VBDs, including mosquito-borne diseases such as malaria (Siraj et al. 2014; Rogers & Randolph 2000b; Gething et al. 2010; Patz & Olson 2006b; Loiseau et al. 2013). Fluctuations in environmental factors connected with climate are expected to affect the spread of malaria at global and local levels (Rogers & Randolph 2000b; H. E. Tonnang et al. 2010; Tonnang et al. 2014; Sutherst 2004), but the current understanding of exactly how and to what extent climatic factors, including temperature, currently affect disease transmission is limited (Githeko et al. 2000; Lafferty 2009; Tonnang et al. 2014; Laneri et al. 2015).

This is due partly to the fact that environmental temperature plays an intricate role in VBD transmission, as it affects many of the mosquito and parasite's life-history traits that contribute to transmission. For example, it is well established that higher mean environmental temperatures can shorten the life span of the *Anopheles* mosquito (Craig et al. 1999; Koella 1991; Dawes et al. 2009), and shorten the development time of the *Plasmodium* parasite within the vector (Blanford et al. 2013; Churcher et al. 2013; Eling et al. 2001; Loiseau et al. 2013), both of which affect transmission (Macdonald 1956; Paaijmans et al. 2013). It has also been confirmed that higher temperatures during the development of mosquito larvae speed up their development and result in smaller adult mosquitoes (this is also applicable to other cold-blooded animals) (Rueda et al. 1990; Horsfall 1972; Depinay et al. 2004; Beck-Johnson et al. 2013; Lyimo et al. 1992). Mosquito size in turn affects other factors important in disease transmission, including fecundity, which influences population density (Lyimo & Takken 1993; Takken et al. 2013; Helinski & Harrington 2011), mating probability (Ng'habi et al. 2005), and blood meal size (Takken et al. 2013; Takken et al. 1998a). The size of the blood meal a mosquito ingests affects its longevity and survival (Alto et al. 2008), and influences the uptake of parasites, thereby having an impact on the probability of infection and transmission (Lyimo & Koella 1992). It has been shown that the mean environmental

temperature and temporal temperature fluctuations around the mean, in addition to other factors including rainfall and humidity, affect *Anopheles* population dynamics (Minakawa et al. 2002; Lardeux et al. 2013). Due to the intricacies of these linked effects, quantifying the mechanisms by which climatic factors affect vector ecology is needed for predictions of vector abundance, distribution, evolutionary fitness, and transmission capability (Semenza & Menne 2009; Paaijmans et al. 2013), and thereby forecasts of malaria transmission, under specific climate scenarios.

In addition, as mentioned in chapter 2, while the influence of temperature has been studied during the development of mosquito larvae (Lyimo et al. 1992; Lyons et al. 2013; Bayoh & Lindsay 2003; Bayoh & Lindsay 2004) and throughout adult life (Bayoh & Lindsay (pers.comm.)), no data currently exist describing how the temperature of *Anopheles gambiae* s.s. immature stages' environment affects the life history traits and reproductive parameters of the adult mosquito. A clearer and more comprehensive insight into these interactions should allow more precise and robust predictions of the effect of temperature on the ecology and population dynamics of the vector of malaria, and thus of malaria transmission.

Further results are presented here, following on from chapter 2, on the effects of the temperature of the larval and adult environments of *An. gambiae* s.s. mosquitoes on size, developmental rates, blood feeding, oviposition, and egg hatching. It was decided to examine these particular parameters as they are some of the most important in influencing mosquito abundance and population dynamics.

2. Methods

2.1. Experimental methods

2.1.1. Larval and adult maintenance and temperature regimes

The *Anopheles gambiae* s.s. mosquitoes were obtained from the colony held at Imperial College London's Silwood Park campus as described in chapter 2 in section 3.1. The larval and adult maintenance regimes, as well as the experimental setup and temperature regimes, are defined in chapter 2, in sections 3.1 and 3.2.

2.1.2. Larval developmental rates and larval size

Larvae were examined every 24 hours for emergence to the next developmental stage (from L1 to L2, L2 to L3, L3 to L4), and the time to pupation was recorded for each individual larva (from L4 to P), as well as the time to emergence of each individual pupa into imago (from P to A).

To measure the size of the larval stages, photographs were taken of 20 larvae chosen randomly every 24 hours and the size of the larvae measured and recorded with the software ImageJ (ImageJ 1.45s for Mac OS X, NIH, USA). Larval body length was measured from the distal tip of the head to the end of the anal segment excluding all antennae, feeding brush, and caudal hair (Manoukis et al. 2006). Larval head width (for instar determination) was measured across the widest part of the head (Figure 3.1). As explained in chapter 2, each larva was reared individually, and therefore each mosquito is considered an individual data point. As the sex of the larvae cannot be identified, it was assumed the ratio of males to females was 1:1.

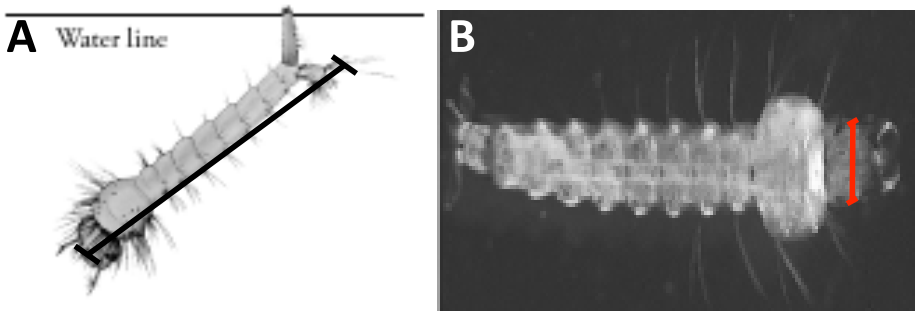


Figure 3.1. *Anopheles* larvae measurement. Larval body is measured from the distal tip of the head to the end of the anal segment, excluding antennae, feeding brush, and caudal hair (Alameda County Mosquito Abatement).

2.1.3. Adult feeding, oviposition, and egg hatching

As described in chapter 2, adults were given four days to mate, following which all males were discarded. The females were then transferred to plastic cups individually and fed with cotton balls soaked with a 10% saturated sugar solution, which was renewed every 48-72 hours, and removed 24 hours before each blood meal to ensure that the mosquitoes were eager to feed. They were then offered blood meals on my arm at three time points: 5, 12, and 19 days after emergence as adults. The time between blood meals was set as 7 days to allow all females to lay eggs and the eggs to hatch. The number of females that fed at each blood meal was counted, and mosquitoes that did not feed were thereafter not included in the experiment. The proportion of females ingesting blood at each blood meal and each of the adult environmental temperatures was documented.

The bottom of each cup was filled with deionised water 24 hours after each blood meal to allow the females to lay eggs, and the mosquitoes were transferred to new, dry cups 72 hours after taking a blood meal. Eggs were kept at the same environmental temperature as their mothers. The number of females that laid eggs after blood feeding, the proportion of egg-

laying females among those which blood fed, the number of eggs laid by these females (a measure of fecundity), and the number of eggs that hatched among those laid (a measure of fertility) were counted. The time between blood feeding and egg laying and the time between egg laying and hatching were also recorded. Censoring occurred 35 days after the emergence of the parental generation into L1 larvae, with all mosquitoes monitored until that day (as described in chapter 2).

2.1.4. Adult female size

On day 35 after the parental generation emerged as L1 larvae, mosquitoes still alive were frozen and all adult females were measured, using wing length as a proxy for size (Lyimo & Takken 1993). To measure adult size, mosquitoes were dissected under a binocular microscope, and the abdomen, thorax, and wings separated. Both wings of each mosquito were removed, fixed onto a microscope slide with clear tape, photographed, and measured from the distal end of the alula to the tip with the software ImageJ (Figure 3.2). As mosquitoes were not followed until their natural death, longer-lasting effects of the temperature of the maternal environment may have been lost.

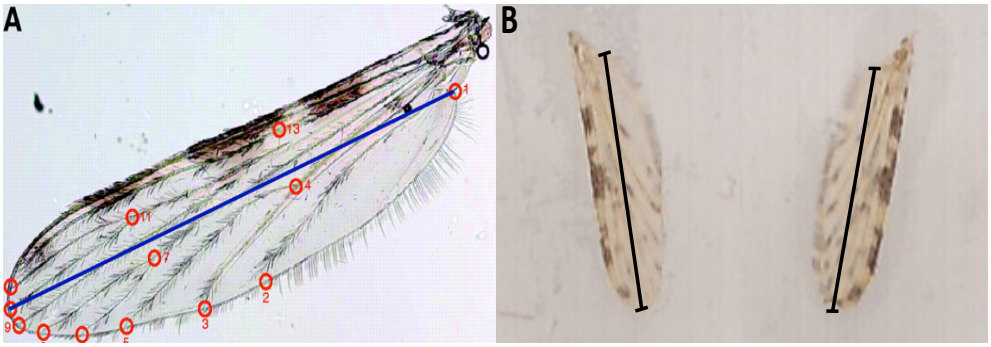


Figure 3.2. Adult *Anopheles* wing measurement. Mosquito wing length is measured from the inside of the wing immediately at the base of the node by which it is attached to the body to the outer tip of the wing. **A**, a wing of *Anopheles gambiae* s.l., with the straight-line wing-length measurement displayed in blue from Huestis et al. 2011. The length of the node is not included in wing-length measurement. **B**, photograph of the wings of an *Anopheles gambiae* s.s. mosquito obtained during training at Imperial College, with the wing length measurement displayed as the black line.

2.2. Statistical methods

2.2.1. Larval developmental rates and larval size

A non-parametric (Kaplan-Meier) survival analysis was performed on the time between each mosquito stage and the next larval, pupal, or adult stage, to compare how the different

immature stages' temperatures affected the time to development from L1 to L2, L2 to L3, L3 to L4, L4 to pupae (P), and pupae to adults (A). The cumulative days to development for the L1–L2, L2–L3, L3–L4, L4–P, and P–A progressions were also calculated.

Tukey's test was used to compare the arithmetic means of instar-specific larval sizes between pairs of larval temperature regimes. For each of the seven days during which the larvae were followed to pupation, the arithmetic mean of larval sizes across all larval stages (from L1 to L4) were compared across all four temperatures using the F statistic.

2.2.2. Blood feeding and oviposition

The influence of larval and adult temperatures on the probability of female mosquitoes blood feeding (given as the proportion of females ingesting blood out of all females that were offered a blood meal at each temperature), and on the probability of females ovipositing after each blood meal (given as the proportion of females ovipositing out of those that had blood fed), was analysed by logistic regression and compared by log-likelihood ratio test.

The mean number of eggs laid by females after each blood meal was analysed by Tukey's test to infer the influence of larval and adult temperatures and temperature combinations on the number of eggs laid by females at each temperature regime. Eggs were kept at the same air temperature as their mothers, and the mean proportion of eggs that hatched at each temperature (23°C, 27°C, and 31°C) was compared by Mann-Whitney test. The mean number of days between each blood meal and egg-laying event, and between oviposition and hatching, were compared by Mantel-Cox test for the pair-wise comparisons between individual temperatures and by log-rank test for the comparison over all temperatures.

2.2.3. Adult female size

Adult wing length was measured as a proxy for body size (Maïga et al. 2012; Kweka et al. 2012; Lyimo & Koella 1992; Artis et al. 2014; Nasci 1990). The length of both wings of each mosquito was measured and the arithmetic mean recorded as the 'size' of each mosquito. A one-way analysis of variance (ANOVA) determined the effect of larval temperature on adult wing length.

3. Results

3.1. The effect of larval temperature on larvae developmental rates and size

For male and female mosquitoes combined, higher temperature from 23°C to 31°C increased the time between hatching and emergence as adults (Figure 3.3). No adults emerged from

pupae at 35°C. The effect of temperature differed across larval stages. Higher temperatures lengthened the development times of first instar larvae and of pupae, but shortened the development times of fourth instar larvae. More precisely, from larval stage L1 to L2, and from the pupal to adult stage, higher environmental temperatures resulted in faster development ($p<0.001$ overall, but no difference was observed in the development times into L2 larvae at 35°C compared with 31°C). An increase of 4°C from 23°C to 27°C decreased development time from the L2 to L3 stage ($p<0.001$), while a further 4°C increase to 31°C resulted in a slower L2 to L3 development time than at 27°C, but a shorter development time than at 23°C ($p<0.001$ in both cases). Increasing larval temperature further to 35°C caused no significant change in development times compared with 31°C ($p=0.238$). From larval stage L3 to L4, and from L4 to the pupal stage, a 4°C rise in temperature, from 23°C to 27°C and from 27°C to 31°C, resulted in an increase in larval development time ($p<0.001$), whereas a 4°C temperature increase from 31°C to 35°C shortened the development time ($p=0.005$). Considering only the female mosquitoes, the age at emergence increased with increasing temperature from 10.92 days at 23°C to 12.35 days at 31°C. The results of the Kaplan-Meier analyses are presented in Figure 3.4.

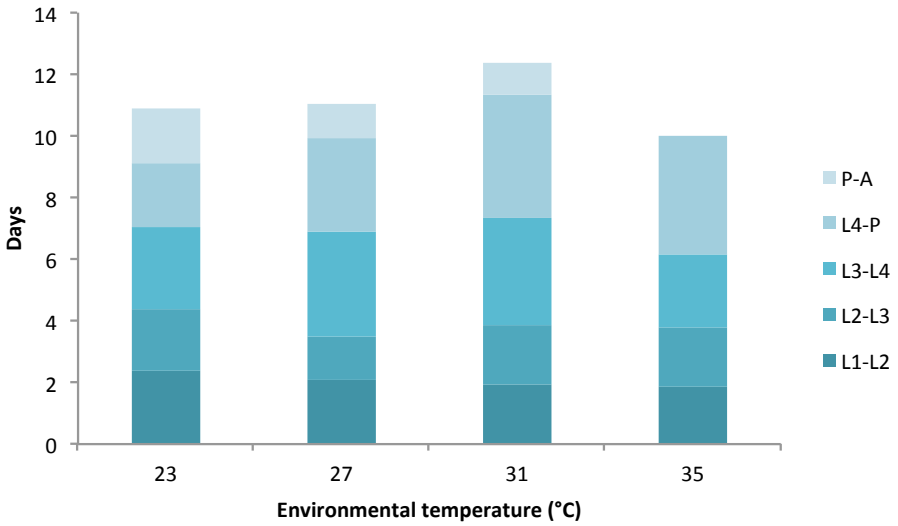


Figure 3.3. Cumulative number of days to development of each life stage in *Anopheles gambiae* s.s.. Larval stages are L1, L2, L3 and L4; pupae are denoted by P and adults by A. Larvae kept at 35±1°C died, so that imagoes did not emerge from their pupal cases at this temperature.

Figure 3.5 shows the daily increase in larval size (averaging across all larvae within one stage, at each larval stage) at all four environmental temperatures for seven days, by which time all larvae achieved pupation. Overall, the temperature of the larval environment had a significant effect on larval size ($p=0.005$). Increasing the environmental temperature resulted in smaller larvae on days 1 ($p<0.001$), 2 ($p<0.001$), 4 ($p<0.001$), 5 ($p=0.006$), 6 ($p<0.001$) and 7

($p<0.001$), while higher temperatures resulted in larger larvae on day 3 ($p<0.001$). The larval sizes, as well as the results of the pair-wise comparisons between mean larval sizes for larvae reared at every temperature regime, and test statistics for the overall comparisons across all temperatures are given in Table 3.1.

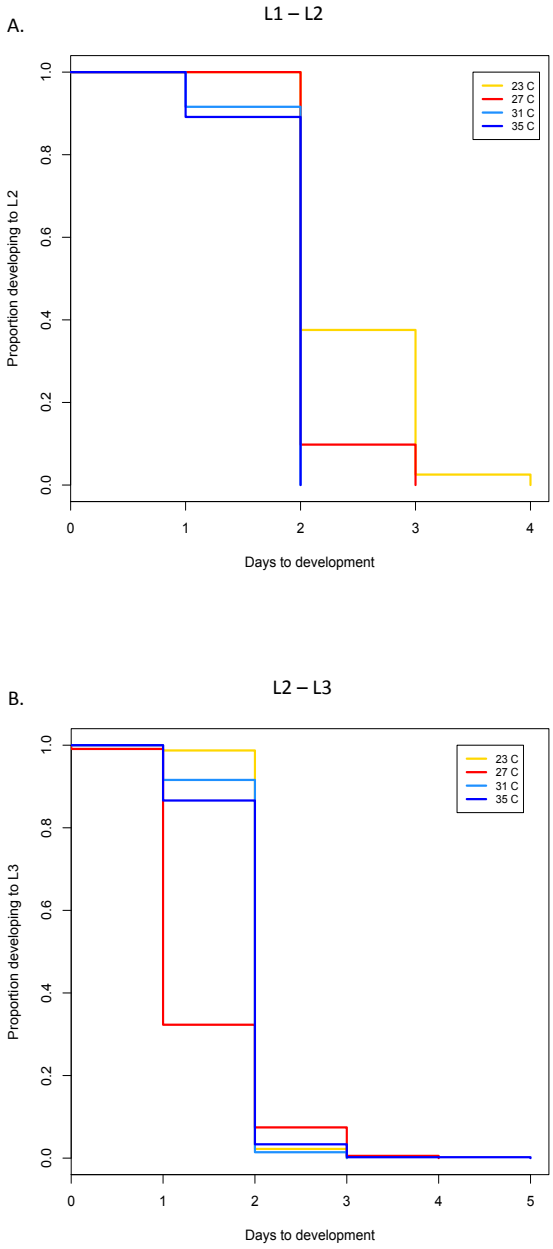


Figure 3.4. Kaplan-Meier plots of the stage-specific survival analysis of development times. A shows the development time from L1 to L2 larvae, and **B** from L2 to L3. Coloured lines represent the environmental temperatures investigated as described in Fig. 3.3.

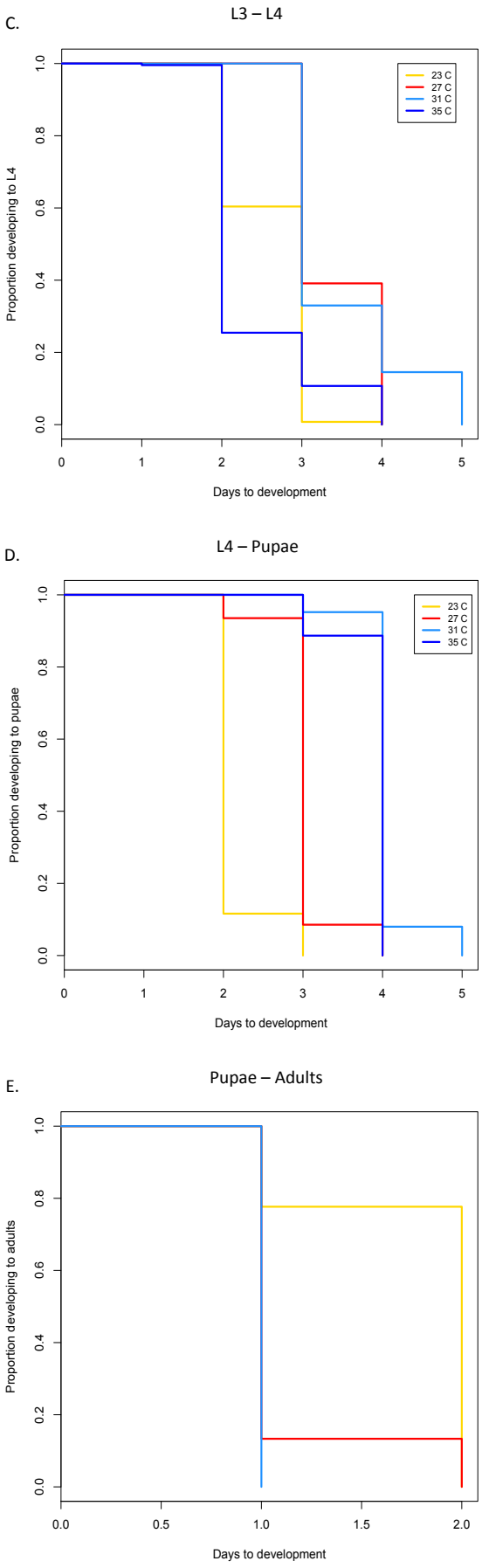


Figure 3.4. **C** shows the development time from L3 to L4, **D** from L4 to pupae, and **E** from pupae to adults. Coloured lines represent the environmental temperatures investigated as described in Fig. 3.3.

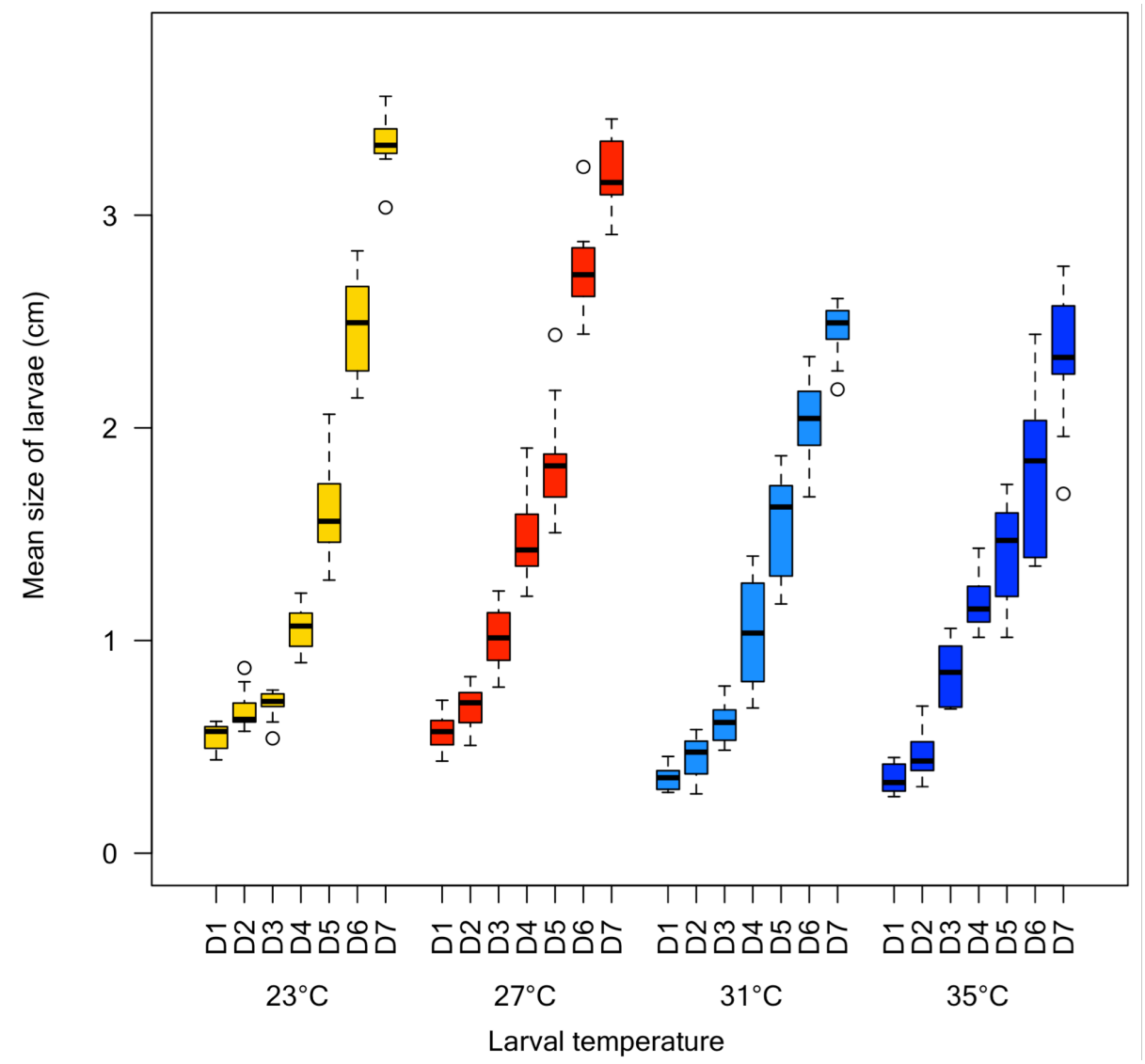


Figure 3.5. Mean larval size by day as a function of environmental temperature. Size of larvae (in cm) by day post-hatching (D1 is 24 hours post-hatching, D2 48 hours post-hatching, etc.) and by temperature (23±1°C: yellow; 27±1°C: red; 31±1°C: light blue; 35±1°C: dark blue). The bottom and top of the boxes describe the first and third quartiles respectively, and the bold line inside the boxes represents the median. The whiskers show the data minima and maxima, and the small circles represent outliers.

Table 3.1. Two-group comparisons and overall trend of the effect of increasing larval environment temperature on mean larval size across all larval instars (L1 to L4) for seven days prior to pupation in *Anopheles gambiae* s.s. (wrt = with respect to). F-statistic for overall effect of temperature on larval body size: 4.43, p-value: 0.005

		23±1°C	**27±1°C (wrt 23°C)**	**31±1°C (wrt 23°C)**	**31±1°C (wrt 27°C)**	**35±1°C (wrt 23°C)**	**35±1°C (wrt 27°C)**	**35±1°C (wrt 31°C)**	**Overall effect on larval body length**	
Day 1	Size (± SE) (mm)	5.47 (0.21)	5.64 (0.29)	3.53 (0.18)		3.50 (0.21)				
	Tukey test statistic		0.24	49.12	38.20	43.90	35.86	0.03	F statistic	27.20
	p-value		0.63	<0.001	<0.001	<0.001	<0.001	0.86	p-value	<0.001
Day 2	Size (± SE) (mm)	6.75 (0.30)	6.91 (0.30)	4.54 (0.31)		4.62 (0.39)				
	Tukey test statistic		0.15	25.93	30.03	18.88	21.95	0.03	F statistic	15.85
	p-value		0.71	<0.001	<0.001	<0.001	0.001	0.87	p-value	<0.001
Day 3	Size (± SE) (mm)	6.99 (0.22)	10.13 (0.47)	6.16 (0.32)		8.45 (0.50)				
	Tukey test statistic		36.73	4.64	49.53	7.15	6.11	15.12	F statistic	19.81
	p-value		<0.001	0.04	<0.001	0.02	0.02	0.001	p-value	<0.001
Day 4	Size (± SE) (mm)	10.54 (0.33)	14.71 (0.68)	10.35 (0.76)		11.87 (0.43)				
	Tukey test statistic		30.26	0.05	18.07	6.12	12.32	3.02	F statistic	12.06
	p-value		<0.001	0.83	<0.001	0.02	0.003	0.10	p-value	<0.001
Day 5	Size (± SE) (mm)	16.24 (0.83)	18.46 (0.88)	15.50 (0.81)		14.15 (0.73)				
	Tukey test statistic		3.38	0.41	6.16	3.56	14.2	1.51	F statistic	4.9
	p-value		0.08	0.53	0.02	0.08	0.001	0.23	p-value	0.006
Day 6	Size (± SE) (mm)	24.83 (0.73)	27.4 (0.70)	20.28 (0.71)		17.85 (1.20)				
	Tukey test		6.58	20.02	51.19	24.82	47.56	3.03	F statistic	25.28
	p-value		0.02	<0.001	<0.001	<0.001	<0.001	0.10	p-value	<0.001
Day 7	Size (± SE) (mm)	33.44 (0.45)	31.86 (0.57)	24.55 (0.43)		23.26 (0.99)				
	Tukey test		4.63	200.10	103.20	86.45	56.00	1.41	F statistic	61.03
	p-value		0.05	<0.001	<0.001	<0.001	<0.001	0.25	p-value	<0.001

3.2. The effect of larval and adult temperatures on blood feeding rates

The temperature at which mosquitoes were reared as larvae had no significant effect on the probability of taking a second blood meal, but females reared at 31°C were significantly less likely to blood feed a third time than at 23°C ($p<0.001$) and 27°C ($p=0.002$) (Table 3.2).

All females blood-fed the first time but the temperature at which adults were kept had a significant effect on the probability of feeding a second and third time (Table 3.3). For the second blood meal, increasing the temperature from 23°C to 27°C and from 23°C to 31°C decreased the probability of feeding from 0.86 to 0.76 ($p=0.049$) and to 0.64 ($p<0.001$) respectively. An increase from 27°C to 31°C did not significantly reduce feeding (0.76 at 27°C vs. 0.64 at 31°C ($p=0.065$)). For the third blood meal, increases from 23°C to 27°C, 27°C to 31°C, and 23°C to 31° all significantly decreased the probability of blood feeding (0.48 vs. 0.22, $p<0.001$; 0.22 vs.0.04, $p=0.002$; and 0.48 vs.0.04, $p<0.001$, respectively).

Table 3.2. Comparison by logistic regression of the effect of larval temperatures on the feeding probability of adults.

	Test statistic	27°C±1 (wrt 23°C)	31°C±1 (wrt 23°C)	31°C±1 (wrt 27°C)
2nd blood meal	Log odds of feeding	0.562	0.45	-0.111
	log-likelihood ratio test	1.803	1.344	-0.322
	p-value	0.071	0.179	0.748
3rd blood meal	Log odds of feeding	-0.194	-1.466	-1.272
	log-likelihood ratio test	-0.673	-3.591	-3.139
	p-value	0.501	<0.001	0.002

Table 3.3 The effect of adult temperature on the feeding probability of adults
Two-group comparisons and overall trend.

	Test statistic	27°C±1 (wrt 23°C)	31°C±1 (wrt to 23°C)	31°C±1 (wrt 27°C)
2nd blood meal	Log odds of feeding	-0.706	-1.272	-0.566
	log-likelihood ratio test	-1.972	-3.509	-1.844
	p-value	0.049	<0.001	0.065
3rd blood meal	Log odds of feeding	-1.188	-2.959	-1.771
	log-likelihood ratio test	-4.004	-5.397	-3.177
	p-value	<0.001	<0.001	0.0015

3.3. The effect of larval and adult temperatures on egg laying, number of eggs laid, and egg hatching

Rearing larvae at higher temperatures significantly decreased the probability of egg-laying for adults kept at 23°C and 27°C, but not for adults kept at 31°C, after the first two blood meals (Table 3.4; all females laid eggs after the third blood meal). The temperature of the adult environment did not affect the probability of females laying eggs after their first or third blood meal whereas after the second blood meal, a temperature increase from 27°C to 31°C and from 23°C to 31°C resulted in significantly lower probabilities of laying eggs (0.65 vs. 0.46 (p=0.022), and 0.72 vs. 0.46 (p=0.002), respectively).

Table 3.5 shows that the temperature at which adult were reared as larvae significantly affected the number of eggs laid at 23°C and 31°C after the first blood meal, but had no effect at 27°C – a result similar to the effect of temperature on the time to oviposition (see below). After the second blood meal, for adults kept at 23°C, females reared at 27°C and 31°C laid significantly fewer eggs than those reared at 23°C. Females reared at 31°C also laid fewer eggs than females reared at 27°C for adults kept at 27°C. The temperature at which females were reared as larvae did not affect the number of eggs laid after the third blood meal.

Table 3.4. The effect of larval temperature on the odds of adult laying eggs. Two-group comparisons and overall trend of the effect of increasing larval environmental temperature on the odds of laying eggs at all adult temperatures for the first two blood meals. All females laid eggs after the third blood meal. [§]A negative value of the log odds represents a decrease in the proportion of females laying eggs with respect to the reference temperature, whilst a positive value[‡] represents an increase in egg laying rate.

			Larval temperature (°C)		
	Adult temperature (°C)		**27±1 (wrt 23°C)**	**31±1 (wrt 23°C)**	**31±1 (wrt 27°C)**
1st blood meal	**23±1**	Log odds of laying eggs	-0.783[§]	-1.658	-0.876
		log-likelihood ratio test	-1.187	-2.455	-1.515
		p-value	0.235	0.014	0.130
	27±1	Log odds of laying eggs	-2.565	-4.069	-1.504
		log-likelihood ratio test	-2.383	-3.828	-3.086
		p-value	0.017	<0.001	0.002
	31±1	Log odds of laying eggs	0.767[‡]	-0.028	-0.795
		log-likelihood ratio test	1.386	-0.048	-1.392
		p-value	0.166	0.962	0.164
2nd blood meal	**23±1**	Log odds of laying eggs	-1.952	-3.312	-1.36
		log-likelihood ratio test	-2.398	-3.805	-2.276
		p-value	0.016	<0.001	0.023
	27±1	Log odds of laying eggs	-1.161	-2.496	-1.335
		log-likelihood ratio test	-1.613	-3.486	-2.534
		p-value	0.107	<0.001	0.011
	31±1	Log odds of laying eggs	1.121	0.359	-0.762
		log-likelihood ratio test	1.557	0.465	-1.218
		p-value	0.120	0.642	0.223

Table 3.5. The effect of larval temperature on the number of eggs laid. Two-group comparisons and overall trend of the effect of increasing larval temperature on the mean number of eggs laid at all adult temperatures. No mosquitoes held at 31°C survived long enough to blood feed for a third time.

	Adult temperature (°C)		**Larval temperature (°C)**			
			23±1	**27±1 (wrt 23°C)**	**31±1 (wrt 23°C)**	**31±1 (wrt 27°C)**
1st blood meal	**23±1**	Mean n° of eggs (±SD)	53.11 (2.66)	41.39 (2.98)	22.31 (5.79)	22.31 (5.79)
		Test statistic (p-value)		2.941 (p<0.05)	5.501 (p<0.01)	3.212 (p<0.05)
	27±1	Mean n° of eggs (±SD)	48.67 (2.86)	52.77 (4.09)	48.35 (5.14)	
		Test statistic (p-value)		-0.846 (0.4)	0.057 (0.95)	0.662 (0.51)
	31±1	Mean n° of eggs (±SD)	39.00 (3.9)	23.17 (2.77)	14.29 (3.38)	
		Test statistic (p-value)		3.337 (p<0.01)	4.719 (p<0.001)	1.899 (0.064)
2nd blood meal	**23±1**	Mean n° of eggs (±SD)	57.53 (3.23)	38.6 (3.55)	30.71 (2.21)	
		Test statistic (p-value)		3.929 (p<0.001)	3.803 (p<0.001)	1.146 (0.261)
	27±1	Mean n° of eggs (±SD)	46.57 (5.57)	57.96 (4.60)	28.17 (8.05)	28.17 (8.05)
		Test statistic (p-value)		-1.584 (0.12)	1.907 (0.065)	3.455 (p<0.01)
	31±1	Mean n° of eggs (±SD)	29.75 (4.23)	13.73 (4.19)	22.00 (5.71)	
		Test statistic (p-value)		1.88 (0.077)	0.932 (0.376)	-1.136 (0.269)
3rd blood meal	**23±1**	Mean n° of eggs (±SD)	50.54 (2.47)	44.6 (2.58)	36.00 (3.96)*	
		Test statistic (p-value)		1.657 (0.1)	2.535 (p<0.05)	1.548 (0.135)
	27±1	Mean n° of eggs (±SD)	54.18 (7.08)	47.20 (6.60)	50.75 (28.21)	
		Test statistic (p-value)		0.76 (0.457)	0.173 (0.866)	-0.186 (0.855)

Figure 3.6 shows the effect of the adult environmental temperature on the number of eggs laid after all three blood meals. After their first blood meal, females kept at 23°C and 27°C laid 43 ± 2 eggs (mean ± SE) and 50 ± 2 eggs (p=0.036 for the comparison between 23°C and 27°C), while females at 31°C laid significantly fewer eggs (25 ± 2 eggs; p<0.001 for both comparisons). After their second blood meal, females at 23°C and 27°C laid 47 ± 2 eggs and 47 ± 4 eggs (p=0.953), while females at 31°C laid 18 ± 3 eggs (p<0.001 for both comparisons). After their third blood meal, females at 23°C and 27°C laid 47 ± 2 eggs and 51 ± 6 eggs (p=0.365), whereas females at 31°C laid 10 ± 7 eggs (p<0.001 when compared with 23°C and p=0.007 when compared with 27°C).

The temperatures at which the mothers were kept as adults had no effect on the mean proportion of eggs that hatched into L1 larvae after the first and third blood meals. After the second blood meal, the mean proportion of eggs laid by females reared at 27°C and 31°C that hatched was significantly higher when compared with the mean proportion of eggs laid by females reared at 23°C that hatched (Table 3.6). This is likely a chance event, as the environmental temperature at which the mothers were reared had no other impact on the mean proportion of eggs that hatched. The temperature at which the adult females were reared as larvae did not influence the proportion of eggs that hatched (Table 3.7).

Table 3.6. Effect of adult temperature on the proportion of eggs hatching after each blood meal.

		23±1°C	**27±1°C (wrt 23°C)**	**31±1°C (wrt 23°C)**	**31±1°C (wrt 27°C)**
1st blood meal	Proportion	0.960	0.964	0.961	
	Test statistic (p-value)		-1 (0.757)	-0.17 (0.690)	0.58 (0.825)
2nd blood meal	Proportion	0.890	0.903	0.742	
	Test statistic (p-value)		-1.63 (0.784)	8.73 (0.007)	9.78 (0.019)
3rd blood meal	Proportion	0.810	0.795	0.632	
	Test statistic (p-value)		1.07 (0.315)	2.76 (0.010)	2.44 (0.043)

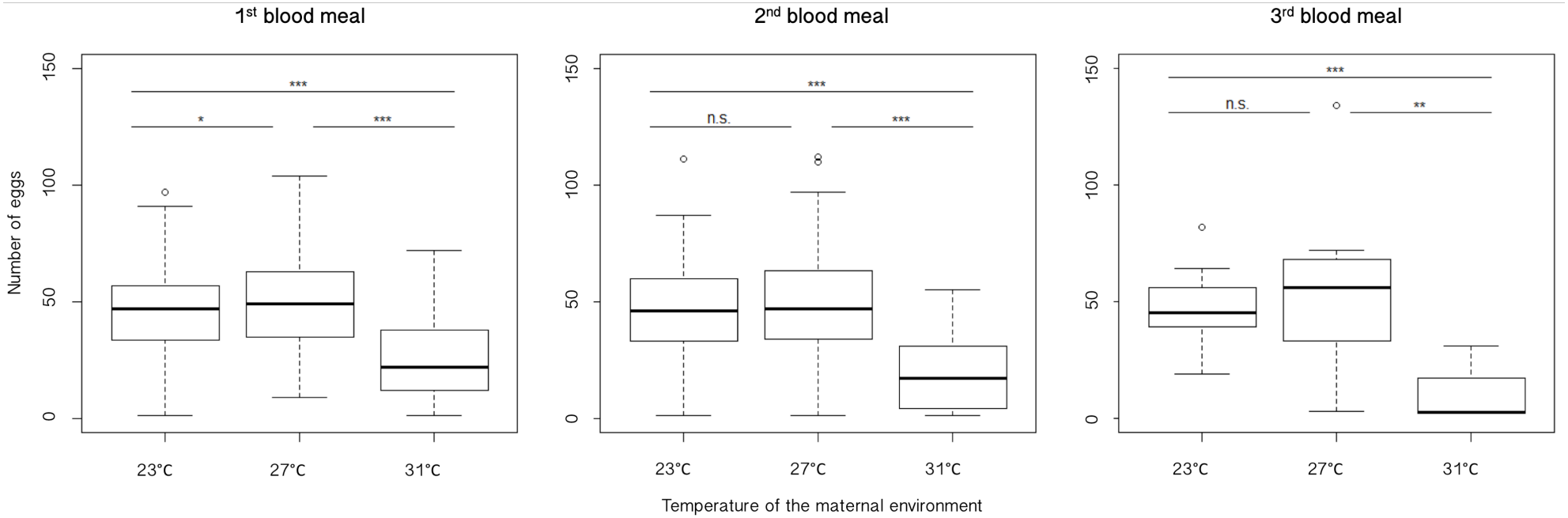


Figure 3.6. Number of eggs laid at each adult temperature after each blood meal. The bottom and top of the boxes describe the first and third quartiles respectively, and the bold line inside the boxes represents the median. The whiskers show the data minima and maxima, and the small circles represent outliers. (n.s.: not significant; *: $p<0.05$; **: $p<0.01$; ***: $p<0.001$)

Table 3.7. The effect of larval temperature on the proportion of eggs hatching. Proportion of eggs that hatched was measured after each blood meal taken by *Anopheles gambiae* s.s. females reared from these larvae and kept at each of the adult temperatures. No mosquitoes survived at 31°C long enough to blood feed for a third time.

			Larval temp (°C)			
	Adult temp (°C)		**23±1°C**	**27±1 (wrt 23°C)**	**31±1 (wrt 23°C)**	**31±1 (wrt 27°C)**
1st blood meal	**23±1**	Proportion	0.964	0.973	0.879	
		Test statistic (p-value)		-1.36 (0.292)	6.31 (0.362)	6.97 (0.831)
	27±1	Proportion	0.958	0.969	0.971	
		Test statistic (p-value)		-1.76 (0.402)	-1.64 (0.806)	-9.94 (0.528)
	31±1	Proportion	0.972	0.963	0.920	
		Test statistic (p-value)		0.97 (0.766)	3.21 (0.336)	2.43 (0.464)
2nd blood meal	**23±1**	Proportion	0.940	0.850	0.637	
		Test statistic (p-value)		7.92 (0.015)	14.59 (0.007)	7.2 (0.242)
	27±1	Proportion	0.882	0.925	0.879	
		Test statistic (p-value)		-3.56 (0.763)	0.17 (0.130)	2.71 (0.078)
	31±1	Proportion	0.485	0.779	0.864	
		Test statistic (p-value)		-5.15 (0.481)	-6.54 (0.217)	-2.04 (0.62)

3.4. Time to egg laying and time to egg hatching

Table 3.8 shows the effect of the environmental temperature at which adult females were reared as larvae on the time between taking a blood meal and laying eggs, after each blood meal. The temperature at which the larvae were kept affected the time to egg laying following the first blood meal only: increasing the environmental temperature of the larvae from 23°C to 27°C and from 23°C to 31°C resulted in a significantly longer time to egg-laying (from 4.03 to 4.15 days ($p=0.03$) and 4.29 days ($p<0.001$), respectively).

Larval temperature had no effect on the time to oviposition after the second (mean duration: 4.0 days) or third blood meals (mean duration: 3.45 days). When the larvae were reared at 23°C, the average time to oviposition was significantly shorter after the third blood meal (3.57 days) than after the first (4.03 days, $p=0.001$) or second blood meal (4.0 days, $p=0.02$). When larvae were reared at 27°C, the

time to egg-laying was also significantly shorter after the third blood meal (3.31 days) compared with the first (4.15 days, $p<0.001$) and second blood meal (4.03 days, $p<0.001$). When larvae were reared at 31°C, time to oviposition was significantly shorter after the second blood meal (4.0 days) and after the third blood meal (3.44 days) when compared with the first blood meal (4.29 days, $p=0.004$ and $p<0.001$ respectively). There was no significant difference between the second (4.0 days) and third blood meal (3.44 days, $p=0.057$).

At all three adult environmental temperatures, the time between blood feeding and egg-laying decreased significantly after each blood meal (23°C: $p<0.001$; 27°C: $p<0.001$; 31°C: $p=0.027$; Table 3.9). However, maintaining adult mosquitoes at warmer temperatures did not affect the time to egg-laying after the first, second, or third batch of eggs (Table 3.10).

Table 3.8. The effect of larval temperature on time to egg-laying. Two-group comparisons and overall trend of the effect of increasing larval environmental temperature on the time to egg laying.

		23°C±1	**27°C±1 (wrt 23°C)**	**31°C±1 (wrt 23°C)**	**31°C±1 (wrt 27°C)**	**Overall effect of larval temperature on time to egg laying**	
1st blood meal	Days (±SD)	4.03 (±0.18)	4.15 (±0.36)	4.29 (±0.46)			
	Mantel-Cox test statistic		4.51	11.08	2.20	Log-rank test statistic	11.06
	p-value		0.03	<0.001	0.14	p-value	<0.001
2nd blood meal	Days (±SD)	4.00	4.03 (±0.18)	4.00 (±0.0)			
	Mantel-Cox test statistic		0.48	0	0.50	Log-rank test statistic	0.05
	p-value		0.49	0.99	0.48	p-value	0.83
3rd blood meal	Days (±SD)	3.57 (±0.5)	3.31 (±0.53)	3.44 (±0.53)			
	Mantel-Cox test statistic		2.02	0.22	0.19	Log-rank test statistic	2.03
	p-value		0.16	0.64	0.67	p-value	0.36

Table 3.9. The effect of adult temperature on the time to egg-laying. Two-group comparisons (Mantel-Cox test) and overall trend (log-rank test) of the effect of increasing adult environmental temperature on the time to laying eggs.

Blood meal		23°C±1	27°C±1 (wrt 23°C)	31°C±1 (wrt 23°C)	31°C±1 (wrt 27°C)	Overall effect of adult temp on time to egg laying	
1st Blood meal	Days (±SD)	4.11 (±0.31)	4.12 (±0.32)	4.18 (±0.39)	4.18 (±0.39)		
	Mantel-Cox test statistic (p-value)		0.02 (0.90)	0.88 (0.35)	0.69 (0.41)	Log-rank test statistic (p-value)	0.84 (0.36)
2nd Blood meal	Days (±SD)	4.00 (±0.0)	4.00 (±0.0)	4.08 (±0.27)	4.08 (±0.27)		
	Mantel-Cox test statistic (p-value)		0.00 (0.99)	1.43 (0.23)	1.37 (0.24)	Log-rank test statistic (p-value)	1.29 (0.26)
3rd Blood meal	Days (±SD)	3.47 (±0.50)	3.46 (±0.51)	3.00 (±0.82)	3.00 (±0.82)		
	Mantel-Cox test statistic (p-value)		0.00 (0.96)	0.90 (0.34)	0.76 (0.38)	Log-rank test statistic (p-value)	0.93 (0.63)

Table 3.10 shows the influence of the environmental temperature of the eggs on the time to hatching. Warmer temperatures significantly quickened the time to hatching of the eggs laid after the first and second blood meals ($p<0.001$ in both cases), although at 31°C there was no effect on the time to hatching of the eggs produced after the third blood meal ($p=0.69$).

For eggs laid after the first blood meal, a 4°C increase in the environmental temperature, from 23°C to 27°C, significantly decreased the time to hatching (1.72 days to 1.07 days ($p<0.001$)), but a 4°C increase from 27°C to 31°C increased the time to hatching (1.07 to 1.75 days ($p<0.001$)). The difference in time to hatching between the coldest (23°C) and the warmest environmental temperature (31°C) was not significant ($p=0.82$).

For the second batch of eggs, an increase of 4°C from 23°C to 27°C significantly reduced the time to hatching, from 2.15 days to 2.11 days ($p<0.001$), while a 4°C increase from 27°C to 31°C did not affect the time to hatching (2.11 days and 2 days respectively, $p=0.91$). An increase of 8°C from 23°C to 31°C also significantly shortened the time to hatching ($p<0.001$).

The temperature at which adult females were reared as larvae had no effect on the time to hatching of the eggs they laid.

Table 3.10. The effect of egg temperature on time to hatching. Two-group comparisons and overall trend of the effect of increasing the environmental temperature of eggs on their time to hatching.

		23±1°C	27±1°C (wrt 23°C)	31±1°C (wrt 23°C)	31±1°C (wrt 27°C)	Overall effect of egg temperature on time to hatching	
1st blood meal	Days (±SD)	1.72 (±0.48)	1.07 (±0.26)	1.75 (±0.81)			
	Mantel-Cox test statistic		61.88	0.05	41.05	Log-rank test statistic	78.49
	p-value		<0.001	0.82	<0.001	p-value	<0.001
2nd blood meal	Days (±SD)	2.43 (±0.65)	1.83 (±0.62)	1.92 (±0.49)			
	Mantel-Cox test statistic		17.71	12.81	0.01	Log-rank test statistic	21.37
	p-value		<0.001	<0.001	0.91	p-value	<0.001
3rd blood meal	Days (±SD)	2.89 (±0.83)	2.45 (±1.1)	3 (±0.0)			
	Mantel-Cox test statistic		0.75	0.03	0.02	Log-rank test statistic	0.75
	p-value		0.39	0.87	0.9	p-value	0.69

3.5. The effect of larval environmental temperature on adult mosquito size

Figure 3.7 shows the mean wing lengths of adult mosquitoes reared at 23°C, 27°C, or 31°C as larvae. There is a significant trend showing that the size of adult female mosquitoes decreases with higher temperatures of the larval environment (overall $p<0.001$). Each 4°C increase in larval temperature resulted in a significant decrease in size (27°C vs. 23°C; 31°C vs. 23°C; and 31°C vs. 27°C; all p-values <0.001; Table 3.11). Unsurprisingly, the temperature of the adult environment had no effect on the wing length of the adult mosquitoes (F-statistic = 0.17, p = 0.844).

Table 3.11. **The effect of larval temperature on adult size**. Two-group comparisons (Tukey test) and overall trend (F-statistic) of the effect of increasing larval environment temperature on the length of adult female wings.

	23±1°C	27±1°C (wrt 23°C)	31±1°C (wrt 23°C)	31±1°C (wrt 27°C)	Overall effect of larval temperature on adult wing length	
Size in mm (± SE)	3.24 (±0.01)	3.12 (±0.02)	2.93 (±0.03)			
Tukey test statistic (p-value)		26.93 (<0.001)	154.57 (<0.001)	38.51 (<0.001)	F statistic (p-value)	65.51 (<0.001)

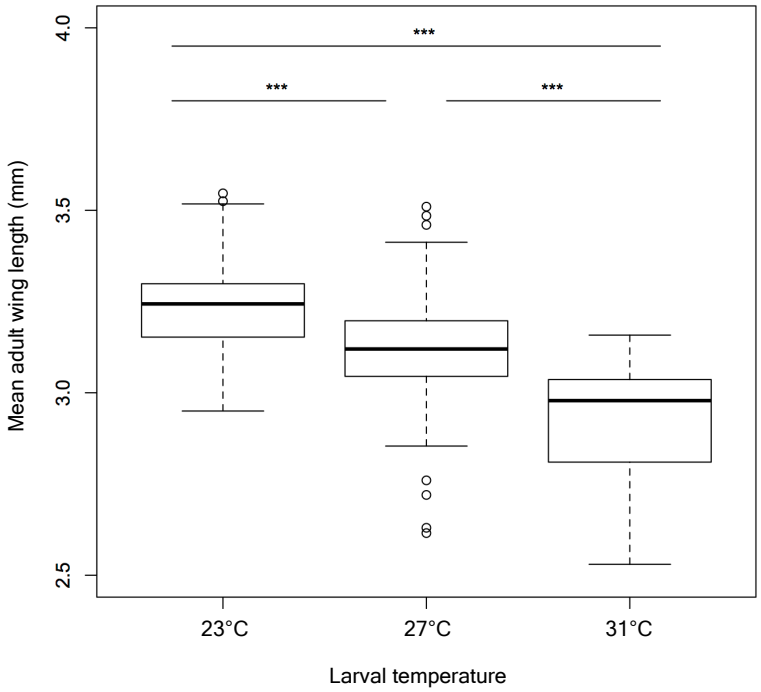


Figure 3.7. Adult wing length (mm) as a function of larval environmental temperature. The bottom and top of the boxes describe the first and third quartiles respectively, and the bold line inside the boxes represents the median. The whiskers show the data minima and maxima, and the small circles represent outliers. (***: $p<0.001$)

4. Discussion

The temperature to which *Anopheles gambiae* s.s. is exposed during all its life stages, from eggs through to adults, is shown in this chapter for the first time to affect several key life-history parameters of considerable importance for a better understanding of the impact of climatic variables on mosquito population dynamics. These results complement those presented in Chapter 2 by examining how the environmental temperature of the immature, aquatic stages, influence not just the survival, but other life-history traits (namely larval and adult size, development rate of each life stage, propensity to blood feed, number of eggs laid (fecundity) and proportion of these that hatch (fertility)) of the adult, aerial stages.

This study confirms results of previous studies (Lyimo et al. 1992; Kirby & Lindsay 2009; Phasomkusolsil et al. 2011) that reported that the larval environmental temperature significantly affect both larval and adult size, and specifically that higher temperatures result in smaller larvae and adults. This has significant ramifications for forecasts of malaria transmission, as *Anopheles gambiae* s.s. size has been shown to play a role in determining its vectorial capacity for malaria spread (Menge et al.

2005). However, there are currently insufficient data on the influence of environmental temperature during both the juvenile and adult stages on the life-history parameters of *Anopheles gambiae* s.s., and this chapter highlights the importance of this lack of information by demonstrating a clear link between the temperature of the juvenile environment and certain adult life-history traits of the mosquito.

In this experiment, the mean time spent in each immature stage was influenced by higher environmental temperature, but the direction of the effect was different for the earlier (decreasing the development time until the L3–L4 stage transition) and later stages (increasing the time from L3–L4 larvae to imagoes), resulting in the net effect that higher temperatures produce longer development times from eggs to imagos. Bayoh and Lindsay (2003) showed that higher temperatures result in increased development rates which peak, in their study, at 28°C. This highlights one of the main limitations of this study: the large increments between the temperature regimes explored here may obscure the behaviour of the mosquito's life-history parameters in the 4°C interval between each temperature documented in this study. Bayoh and Lindsay (2003) also noted that no pupae developed into adults at temperatures above 34°C, which agrees with the results discussed here, which show that larvae and pupae reared at 35°C died before they could emerge as adults.

The data presented in this chapter also hint that there may be an optimal temperature for the fecundity of *Anopheles gambiae* s.s. Adults kept at 27°C emerging from immatures that were kept at 27°C laid the most eggs, whereas those reared and kept at higher and lower temperatures laid significantly fewer eggs. It is noteworthy that the temperature at which females had been reared as larvae also affected the number of eggs laid after the first two blood meals, indicating that differences in numbers of eggs laid was not only due to adult size. Higher adult temperatures resulted in a lower probability of adult females taking blood meals. Where environmental temperatures had an effect on fecundity, higher temperatures also reduced the probability of females ovipositing.

Overall, higher adult temperature did not affect the time between blood meals and oviposition, but the duration of the gonotrophic cycle decreased significantly with each additional blood meal at each temperature. The environmental temperature at which eggs were kept did not result in a perceptible trend in the times between oviposition and hatching, which confirms another study (Impoinvil et al. 2007) and indicates that while temperature may affect the time it takes eggs to hatch, this effect is presumably negligible within an optimal range for development (24-30°C), which is likely to include the temperatures considered here.

Previous research has investigated the link between temperature and the development rate of other *Anopheles* species (Lyimo et al. 1992; Lyons et al. 2013; Phasomkusolsil et al. 2011; Aytekin et al. 2009; Paaijmans et al. 2013), and between temperature and other mosquito vectors (Reiskind & Zarrabi 2012). Other studies have also looked into the influence of temperature on the length of the

gonotrophic cycle, on fecundity, on hatching rates, and on sex ratio (Phasomkusolsil et al. 2011; Lardeux et al. 2008), in addition to the relationship between the quantity of food larvae consume and several life-history parameters (Araújo et al. 2012). The results presented here agree with another study (Impoinvil et al. 2007) that demonstrated that temperature can affect the time to hatching as well as the number of eggs laid.

As highlighted in Chapter 2, the experimental design of this study did not account for the diurnal and nocturnal fluctuations in temperature and humidity that are likely also to influence the development and survival of mosquitoes in the field. In addition, only four larval and three adult temperatures were explored, with 4°C increments, so that some complexities of the mosquito behaviour may have been lost. Future investigation into the life history of *An. gambiae* may determine the manner in which the mosquito reacts to temperatures in between the ones investigated here, particularly to define whether thresholds exist at which the vector's life history and reproductive traits drop off and cease to perform in a continuous fashion. Further research is also needed to investigate the effects of other climatic and environmental factors on *An. gambiae* reproductive and life-history parameters, particularly on developmental rates and on the overall egg yield of the gonotrophic cycle, and to examine the effect of local air temperature fluctuations on the water temperature of mosquito breeding sites (Huang et al. 2006; Paaijmans et al. 2010).

There is currently considerable uncertainty around how temperatures and temperature fluctuations impact the population dynamics of disease vectors. An understanding based on empirical data of how short- and long-term temperature variations will affect the life-history parameters of disease vectors and influence their population dynamics and geographical spread is necessary for more robust and dependable forecasts of disease transmission. To this end, the results of the experimental work described in Chapters 2 and 3 will be used in the following chapters to parameterise mathematical models of *Anopheles gambiae* s.s. population dynamics to mosquito abundance data.

CHAPTER 4

CONSTRUCTION OF A VECTOR MODEL SUITE WITH TEMPERATURE- AND AGE-DEPENDENT MORTALITY

1. Background and Motivation

Mathematical modelling is a very useful instrument allowing us better to understand the transmission dynamics of infectious diseases, including VBDs such as malaria, and better to target and predict the results of control strategies and elimination efforts (Eckhoff et al. 2014; Smith et al. 2006). Mathematical models enable epidemiologists and policy makers to synthesise complex mechanisms (e.g. the parasite's lifecycle and/or the vector's life history parameters), the details and parameters of which may not be accurately known, into one comprehensive picture (Koella 1991). Models of VBDs must account for the part the vectors play in disease spread, by modelling a vector element either explicitly or implicitly (Reiner et al. 2013; Pandey et al. 2013). Where the vector element is explicit, it should depict vector population dynamics as realistically and parsimoniously as possible with respect to the vector biology and ecology (Martens 1998; Rogers 1994; Struchiner et al. 2006; Beck-Johnson et al. 2013).

It has become more universally recognised that climate may affect the spread of VBDs, including mosquito-borne diseases, by influencing vector ecology, and this has led to a surge in experimental research aiming to define the degree to which vector parameters (Chang et al. 2007; Mills et al. 2010; Farjana et al. 2012; Lyons et al. 2013; Couret & Benedict 2014) and vector competence (defined as the capacity to transmit diseases) (Mourya et al. 2004; Mills et al. 2010; Lambrechts et al. 2011; Moller-Jacobs et al. 2014) are dependent on climate-related variables such as temperature, rainfall, and desiccation. It is anticipated that climate will affect vector survival (Ciota et al. 2014) in particular, as this is one of the most important parameters of VBD transmission (Macdonald 1956; Garrett-Jones & Shidrawi 1969; Kurtenbach et al. 2006; Brady et al. 2013), but also developmental rates, reproductive fitness, and behavioural and feeding patterns (Ciota et al. 2014; Gage et al. 2008). All these factors affect the population dynamics of the vector, as well as its seasonal abundance and geographic distribution. Given the increasing evidence that weather-related factors affect the vector and hence transmission dynamics, incorporating the role of climatic elements, and particularly the role of temperature, in models of vector populations and VBDs is becoming more common (Azil et al. 2010; Beck-Johnson et al. 2013; Bessell et al. 2013; Gething et al. 2011; Lunde et al. 2013; Morin & Comrie 2013; Tran et al. 2013; Roiz et al. 2014; Talla et al. 2014).

Modelling the biology and ecology of disease vectors methodically depends upon the availability of exhaustive entomological data to inform the structure and parameterisation of the model (Rogers 1994; Smith et al. 2012; Chabot-Couture et al. 2014; Hollingsworth et al. 2014). An absence of exact and sufficient data may compromise or limit the precision and therefore the value of model results if the model must rely too much on parameter fitting, especially when estimating the value of crucial parameters (Rumisha et al. 2014; Crespo-Pérez et al. 2013). Data on the entomological processes that contribute to vector abundance are in consequence essential for dependable model forecasts.

Current research shows evidence that the mortality of certain disease vectors depends not only on temperature but also on age (Styer, Minnick, et al. 2007; Styer, Carey, et al. 2007; Harrington et al. 2008; Sylvestre et al. 2013). However, this hypothesis may only be valid in a laboratory context: it is unknown whether mosquitoes in the wild survive long enough for age to become an important cause of mortality, or whether the impact of climatic factors on mortality may dwarf the role of senescence (MacDonald 1952; Alto et al. 2014). Most models of VBDs assume constant, age-independent vector mortality, but it has been hypothesised that the more realistic assumption of age-dependent survival may better fit data of observed abundance (Clements & Paterson 1981; Dawes et al. 2009; Hancock et al. 2009; Bellan 2010; Arifin et al. 2014). The manner in which to include age-dependent mortality rates is likely to depend on the vector species, and on biological parameters of the vector, such as feeding patterns and encounters with parasites, pathogens, and insecticides (Takken & Koenraadt 2013).

The suite of four mathematical models presented here is based upon and supported by the experimental work presented in Chapter 2, showing that the main vector of malaria in humans on the African continent, *An. gambiae* s.s., does senesce, and that the survival of *Anopheles gambiae* depends on temperature and age, both in the larval and in the adult stages. This experimental work, which defines the commonly used assumption of time-independent survival as not representative of the mosquito's biology, may have a heavy influence on model design as well as model output: the models developed here are designed to examine how this new experimental result can be included in mathematical models of the malaria vector, and what impact this may have on model development and model predictions. In addition, as the results presented in Chapter 3 show that several reproductive parameters of *An. gambiae* depend unequivocally on environmental temperature, mosquito reproduction is designed here specifically to incorporate the work presented in Chapter 3 on the influence of temperature on the fecundity and hatching rates of *An. gambiae*. In this manner, the models in this chapter are entirely motivated by and based on experimental evidence that may result in improved representations of *Anopheles* population dynamics.

2. Model development and parameterisation

The lifecycle of *Anopheles gambiae* consists of four main stages: egg, larval, pupal, and adult stages. The egg, larval, and pupal stages are aquatic, and only upon emergence from pupae into adults do mosquitoes leave the water. The egg and pupal phases are very short, while larval and adult phases last much longer (the larval stage comprises four sub-stages called instars), although the durations are affected by temperature (Bayoh & Lindsay 2003; Bayoh & Lindsay 2004) see also section 3.3 in Chapter 3). Modelling each stage explicitly is advantageous, partly because there may be considerable variation between development parameters at each stage, and therefore the effect of external elements on these parameters may vary widely, and partly because this allows the inclusion of stage-specific interventions (e.g. larval source management (LSM) for the immature stages, or indoor residual spraying (IRS) for adult females) (Bukhari et al. 2013; White et al. 2011). In the models developed here, all four lifecycle stages are modelled explicitly: the four larval instars are grouped into one larval stage due to the absence of instar-specific data (L), while the egg (E), pupal (P), and female adult (A) stages are modelled separately. The four state variables ($E(t)$, $L(t)$, $P(t)$ and $A(t)$) represent the number of mosquitoes within each stage at time t.

Four models were developed to test the hypothesis that including age-dependent survival in *Anopheles gambiae* population models fits observed longitudinal field abundance data better than modelling survival as time-independent. The first, baseline model, is a simple ODE model with exponentially distributed survival times for eggs, larvae, pupae, and adults, in which mortality within stages depends only on temperature, and is based on the model by White et al. (2011). In the subsequent models, this framework is built upon to include age-dependent survival in the model structure at different stages in the mosquito's life history: in the second model, larval mortality only is assumed to depend on age as well as temperature, and in the third, adult mortality only depends on temperature and age. The final model combines the age-dependent larval mortality of model 2 with the age-dependent adult mortality of model 3. The mortality of the eggs and pupae is set as age-independent in all models, as it was assumed mosquitoes do not remain in these stages long enough to be affected by senescence, and there exists no experimental data to suggest the contrary. While vector survival is often modelled as temperature-dependent, this is the first time that senescence is taken into account.

2.1. The baseline model and parameters

The four variables in all the models developed here are equivalent to the four mosquito stages: eggs (E), larvae (L), pupae (P), and adults (A).

On average, a temperature-dependent proportion $q(T_A)$ of female adults lay a number of eggs $n(T_A)$, of which a proportion $\rho(T_A)$ hatch (these proportions are based on the experimental results in Chapter 3 and depend on the environmental (air) temperature T_A). Eggs either suffer a fixed, temperature-independent daily mortality rate μ_E, or progress to the larval stage at a daily rate $\sigma_E(T_W)$, defined as the inverse of the egg stage duration: $1/d_E(T_W)$ (Table 4.1) (Parham et al. 2012) by Bayoh and Lindsay (unpublished data).

Table 4.1. Average stage duration. Average duration (in days) of egg, larval, and pupal stages at water temperature *Tw* according to Parham et al. (Parham et al. 2012), with corrected coefficients.

Parameter	Functional form for average stage duration
d_E (eggs)	$\left(1.011+\frac{20.212}{1+\left(T_W/12.096\right)^{4.839}}\right)$
d_L (larvae)	$\left(8.13+\frac{13.794}{1+\left(T_W/20.742\right)^{8.946}}\right)-\left(1.011+\frac{20.212}{1+\left(T_W/12.096\right)^{4.839}}\right)$
d_P (pupae)	$\left(8.56+\frac{20.654}{1+\left(T_W/19.759\right)^{6.827}}\right)-\left(8.13+\frac{13.794}{1+\left(T_W/20.742\right)^{8.946}}\right)-\left(1.011+\frac{20.212}{1+\left(T_W/12.096\right)^{4.839}}\right)$

Larvae are exposed to either a temperature-dependent, age-independent daily death rate $\mu_{Lc}(T_A)$ in Models 1 and 3, or a temperature- and age-dependent daily death rate $\mu_L(T_A)$ in Models 2 and 4. They are also subjected to density-dependence, given as a further daily death rate μ_K. Larvae that do not die become pupae at a rate $\sigma_E(T_W)$, given as the inverse of the larval stage duration (Table 4.1) (Parham et al. 2012). Pupae either die at a fixed, temperature-independent daily death rate μ_P or progress to adults at rate $\sigma_P(T_W)$, given as the inverse of the pupal stage duration (Table 4.1) (Parham et al. 2012). It is assumed that half of all the pupae that develop into adults are females (Kirby & Lindsay 2009), and only females are modelled explicitly. Adult females either die at a temperature-dependent, but age-independent, daily death rate $\mu_{Ac}(T_A)$ in Models 1 and 2, or at a temperature- and age-dependent daily death rate $\mu_A(T_A)$ in Models 3 and 4. Parameter values are given in Table 4.2, along with their original reference.

The mean number of eggs laid per female and the proportion of eggs from each batch (i.e. after each blood meal) that hatch were both estimated from data in Chapter 3. To quantify temperature-dependent fertility, the mean number of eggs laid by all females was averaged across all three blood meals at each temperature, and a functional form was fitted to the data points (Figure 4.1, the functional form is

given in Table 4.2). To quantify temperature-dependent hatching, the mean proportion of eggs that hatched from each batch was averaged across all blood meals at each temperature, and a functional form was fitted to the data (Figure 4.2, with the functional form given in Table 4.2).

For those parameters estimated by fitting the models to data (see Chapter 5), the prior values are used as initial values: the prior for the proportion of females laying eggs ($q(T_A)$) was informed by the experimental data presented in Chapter 3, and the priors for the egg mortality rate, the days of rainfall contributing to carrying capacity, and the difference between air and water temperature were informed by the literature). The asterisks indicate values obtained from the posterior distribution determined by fitting the models to surveillance datasets in Chapter 5.

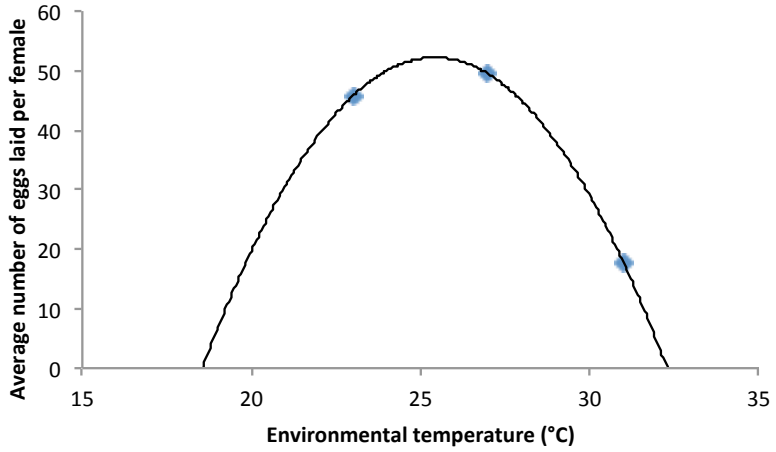


Figure 4.1. Average number of eggs laid as a function of temperature. The blue points show the data from Chapter 3: the average number of eggs laid by all females after all blood meals at each temperature (23°C, 27°C, and 31°C). The black line is the functional form fitted to the data.

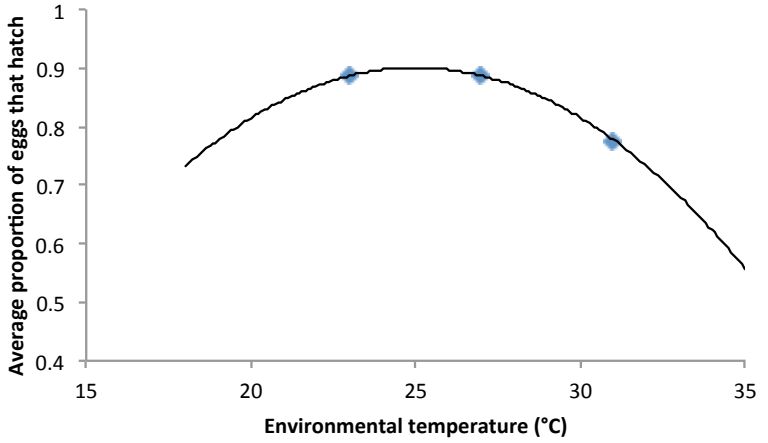


Figure 4.2. Proportion of eggs that hatch as a function of temperature. The blue points are the data obtained in Chapter 3, showing the average proportion of eggs that hatched after all blood meals at each temperature, and the black line is the functional form fitted to the data.

Table 4.2. Model parameters and parameter values. Parameters marked * were inferred, parameters marked ♦ were obtained from data presented in chapter 3.

Parameter	Definition	Unit	Prior	Posterior
q	Proportion of adult females laying eggs	_	0.61-0.85 ♦	*
$n(T_a)$	Number of eggs laid per female	_	$-1.1057Ta^2 + 56.208Ta - 662.1$ ♦	
$\rho(T_a)$	Proportion of eggs hatching	_	$-0.0034Ta^2 + 0.1719Ta - 1.248$ ♦	
μ_E	Per capita egg mortality rate	$days^{-1}$	0.32-0.8 (Lutambi et al. 2013; Okogun 2005)	*
μ_L	Per capita age-dependent larval mortality rate	$days^{-1}$	$1/\alpha_L\beta_L$	
μ_{Lc}	Per capita age-independent larval mortality rate	$days^{-1}$	$0.0013Ta^2 - 0.0704Ta + 0.9581$ ♦	
μ_P	Per capita pupal mortality rate	$days^{-1}$	0.25 (White et al. 2011)	
μ_A	Per capita age-dependent adult mortality rate	$days^{-1}$	$1/\alpha_A\beta_A$	
μ_{Ac}	Per capita age-independent adult mortality rate	$days^{-1}$	$(5.37\text{x}10^{-5})e^{0.228Ta}$ ♦	
μ_K	Per capita density- (and rainfall-) dependent larval mortality rate	$days^{-1}$	$\mu_C \sum_{i=1}^{7} L_i \Big/ K$ (White et al. 2011)	
μ_C	Constant	$days^{-1}$	$0 - 10000$	*
K	Carrying capacity	-	(White et al. 2011)	
τ	Days of rainfall contributing to carrying capacity	days	<10 (White et al. 2011)	*
α_L	Shape parameter of larval gamma hazard function	_	7	
β_L	Scale parameter of larval gamma hazard function	days	$-0.0112Ta^2 + 6.0775Ta - 6.709$ ♦	
α_A	Shape parameter of adult gamma hazard function	_	3	
β_A	Scale parameter of adult gamma hazard function	days	$171.26e^{-0.1191Ta}$ ♦	
σ_E	Per capita egg development rate	$days^{-1}$	$1/d_E$ (Parham et al. 2012)	
σ_L	Per capita larval development rate	$days^{-1}$	$1/d_L$ (Parham et al. 2012)	
σ_P	Per capita pupal development rate	$days^{-1}$	$1/d_P$ (Parham et al. 2012)	
σ_A	Per capita adult development rate	$days^{-1}$	$1/d_A$ (Parham et al. 2012)	
ΔT	Difference between environmental air and water temperature	°C	2.9 – 7.6(Paaijmans et al. 2008)	*

In the four models presented here, relative humidity (RH) is not explicitly included, despite there being some evidence that low levels of humidity can affect adult mosquito longevity (Yamana & Eltahir 2013; Warrell & Gilles 2002). However, an additional density-dependent death rate only affects the larval stage: mosquitoes are estimated to spend too little time, comparatively, in the other two immature stages (eggs and pupae) for density to have a significant impact (Gilpin & McClelland 1979; Legros et al. 2009). The additional hazard among the larvae accounting for density-dependent mortality ($\mu_K(T)$) was derived from White et al. (White et al. 2011) as,

$$\mu_{K(t)} = \mu_c \left(\frac{\sum_{i=1}^{7} L_i}{K(t)} \right), \tag{4.1}$$

where μ_C is a free parameter whose value is determined by model fitting (see Chapter 5), and quantifies the extent to which larval mortality depends on the density of larvae in the breeding site, and $K(t)$ is defined as the carrying capacity of the environment at time t, and is based on the form that best fit the model of White et al. (2011) to datasets from the Garki project (Molineaux & Gramiccia 1980):

$$K(t) = \frac{1}{\tau\left(1 - e^{-1/\tau}\right)} \int_0^t e^{-(t-t')/\tau} rain(t')\,dt\,. \tag{4.2}$$

Given this parameterisation, Model 1 assumes that mortality at all stages depends only on temperature and hence the time spent in each stage is exponentially distributed.

In Model 1 (Figure 4.3 and equation (4.4)), the number of eggs (E) generated and entering the Egg compartment by the proportion, $q(T_A)$, of adults (A) alive who can lay eggs, the number of eggs they lay, $n(T_A)$, and the proportion of eggs that will hatch $\rho(T_A)$. Eggs leave the Egg compartment either by dying, at a fixed daily rate, μ_E, or by progressing to the larval stage at a temperature-dependent daily rate $\sigma_E(T_W)$. Larvae (L) leave the larval compartment either by dying at a temperature-dependent daily death rate $\mu_{Lc}(T_A)$, or at a density-dependent fixed daily death rate μ_K, or by progressing to the pupal stage at a temperature-dependent daily rate $\sigma_L(T_W)$. Pupae (P) leave the pupal compartment either by dying at a fixed daily rate, μ_P, or by progressing to the adult stage at a temperature-dependent daily rate $\sigma_P(T_W)$. Adults (A) can leave the adult compartment only by dying at a temperature-dependent daily death rate $\mu_{Ac}(T_A)$.

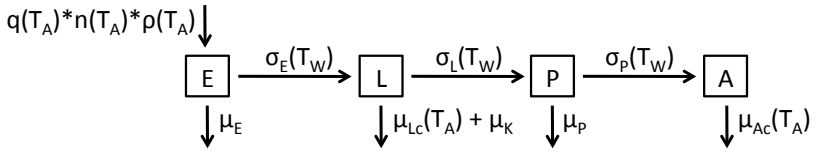


Figure 4.3. **Model 1**. Flow diagram representing the structure of Model 1.

$$\begin{aligned}
\frac{dE}{dt} &= q(T_A)n(T_A)\rho(T_A)A - \left(\mu_E + \sigma_E(T_W)\right)E,\\
\frac{dL}{dt} &= \sigma_E(T_W)E - \left(\mu_{Lc}(T_A) + \mu_E + \sigma_L(T_W)\right)L,\\
\frac{dP}{dt} &= \sigma_L(T_W)L - \left(\mu_P + \sigma_P(T_W)\right)P,\\
\frac{dA}{dt} &= \frac{\sigma_P(T_W)P}{2} - \mu_{Ac}(T_A)A.
\end{aligned} \tag{4.3}$$

2.2. Models including age-dependent survival

The experimental evidence that mosquitoes senesce (Clements & Paterson 1981; Dawes et al. 2009), suggests that the common assumption that mortality rates are constant may be oversimplified, as it does not allow for the fact that as mosquitoes age and experience more gonotrophic cycles, they are more likely to die. Laboratory evidence presented in Chapter 2 supports the claim that survival times are not exponentially distributed, and therefore a constant hazard may not accurately represent the survival of *An. gambiae.* In Chapter 2, four parametric forms for the survivor function $S(t)$ (exponential, gamma, Weibull and Gompertz) were fitted by MLE to laboratory mortality data at four temperatures (23°C, 27°C, 31°C, and 35°C), and the fits compared using their AIC values.

In this chapter, the constant (time-independent) hazard, obtained by fitting the exponential model of $S(t)$ to the survival data, was fitted to the laboratory mortality data (Figures 4.4 and 4.5, blue lines) and compared to the gamma distribution fitted to the laboratory survival data in Chapter 2 (Figures 4.4 and 4.5, red lines). The dependence on temperature of the hazard functions $\mu_{Lc}(T_A)$ and $\mu_{Ac}(T_A)$ was plotted and a functional form fitted (Figure 4.6 and Table 4.2) to define functional forms describing the larval and adult age-independent mortality rates ($\mu_{Lc}(T_A)$ and $\mu_{Ac}(T_A)$ respectively).

At every temperature, and for both larvae and adults, the exponential model fit the data significantly worse than the other models, which suggests that age-independent survival models are less suitable to describing the survival of *An. gambiae* than age-dependent models.

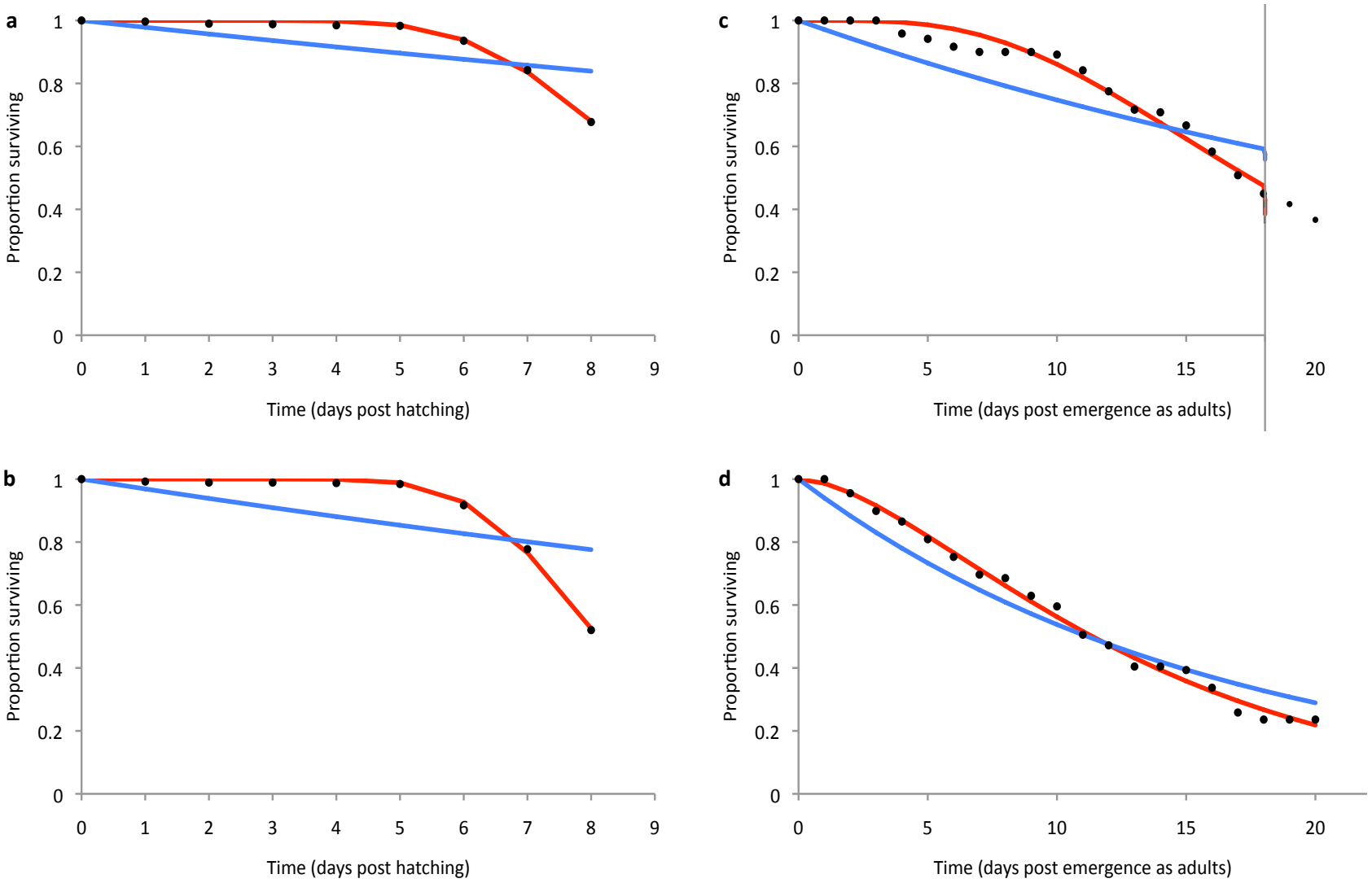


Figure 4.4. Survival function fits. Fit of exponential (blue lines) and gamma (red lines) survival function $S(t)$ to the laboratory survival data in Chapter 2 at 27°C (**a** and **c**) and 31°C (**b** and **d**). **a** and **b** show larval survival, and **c** and **d** are for adults.

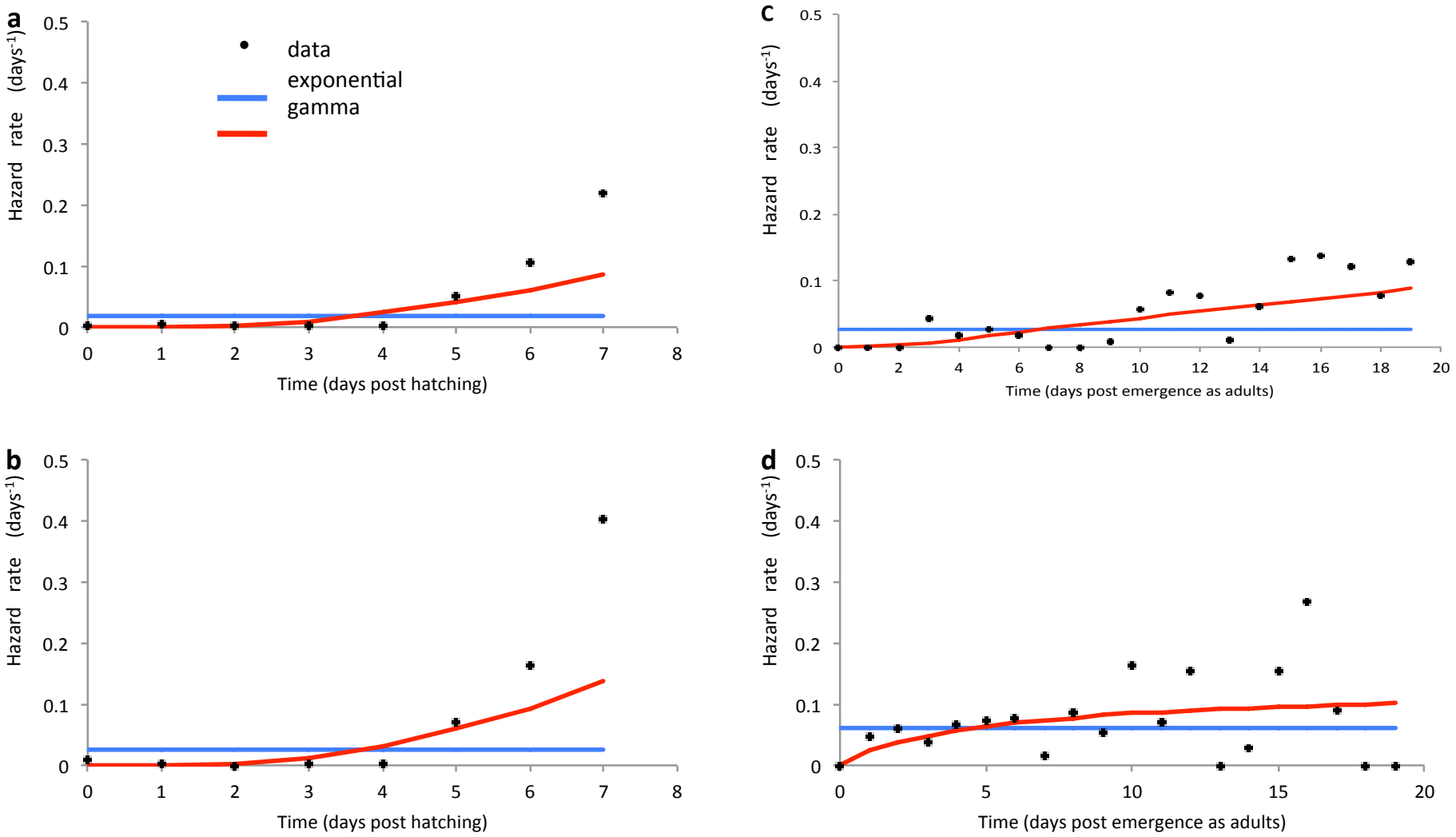


Figure 4.5. Hazard function fits. Fit of constant (blue lines) and time-dependent (red lines) hazard functions to the laboratory data in Chapter 2 at 27°C (**a** and **c**) and 31°C (**b** and **d**). **a** and **b** show larval survival, **c** and **d** are for adults. The constant hazard corresponds to the exponential model, and the age-dependent hazard corresponds to gamma-distributed survival times.

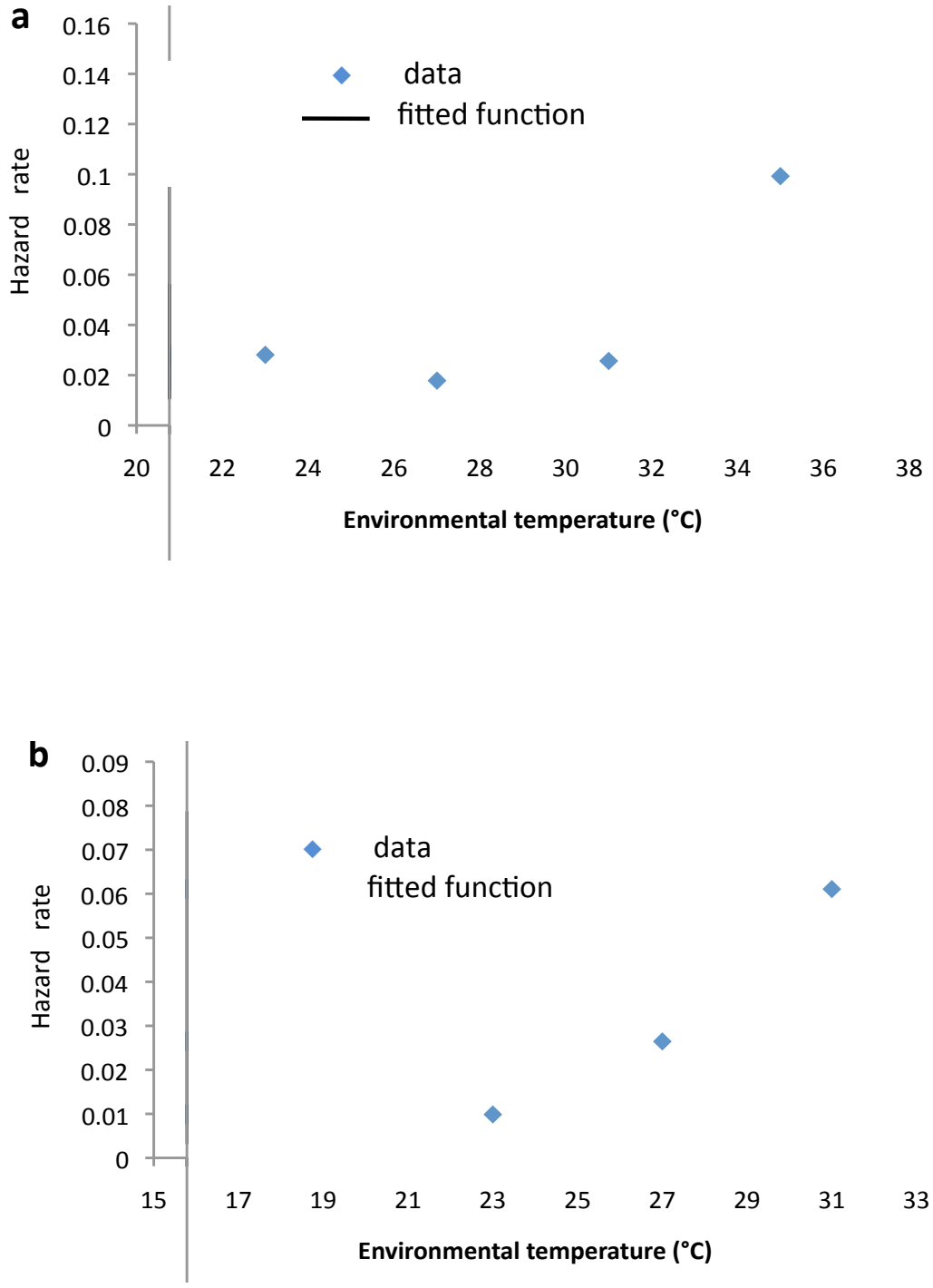


Figure 4.6. Temperature-dependent mortality rates. Age-independent larval (**a**) and adult (**b**) mortality rate as a function of environmental temperature (the best-fit functional forms are given in Table 2).

Although in Chapter 2 the Gompertz distribution fit the data best, the gamma function was chosen to describe survival in this chapter as (a) it also fit the data significantly better than the exponential function at all temperatures, and it was not considerably worse than the Gompertz function in the majority of cases (according to their AIC values, see Table 2.1 in Chapter 2), and (b) the gamma distribution is mathematically and computationally convenient as it can be broken up into more compartments with exponentially distributed waiting times in each compartment (Wearing et al. 2005). In addition, the gamma distribution encompasses a wide range of survival times, and can therefore be useful as a probability distribution for representing survival data, as its non-monotonic time function allows modelling of accelerated failure time (Collett 2003).

For these reasons, age-dependent survival is modelled in this chapter using a gamma distribution *g(t)*, with shape parameter α and scale parameter β:

$$g(t) = \frac{\beta^{\alpha} t^{\alpha-1} e^{-\beta t}}{\Gamma(\alpha)} \tag{4.4}$$

where $\Gamma(\alpha)$ is the gamma function evaluated at α.

The gamma probability distribution has two parameters, both of which fluctuate with temperature in the case of the data presented in Chapter 2. The shape parameter (here given the letter α) described the number of subclasses into which the larvae or adults are divided (Wearing et al. 2005; Lloyd 2001), therefore it was necessary to assign α a fixed value, independent of the environmental temperature. In order to determine the integer value of α that generated the closest fit of the gamma distribution to the MLE-defined best-fit gamma distribution at the larval and adult stages, integer values either side of the original values of α obtained by MLE were assigned to α.

The scale parameter, defined here as β, of the gamma distributions was defined next by fixing the value of α to the integer value obtained above, and fitting β by MLE to the survival data from Chapter 2; the fit of the resulting gamma function was compared to the original best-fit distribution by comparing AIC values. The integer value of α for which the sum of the differences between AIC values across all larval or adult temperatures was minimised was chosen as the fixed value of α for that stage (Tables 4.5 and 4.6). For the larval stage, α was set to 7, and for the adult stage, α was set to 3.

Tables 4.5 and 4.6 show the integer values of α alongside the original best-fit MLE-determined values of α at different temperatures for the larval and adult stages respectively; also shown are the differences between the AIC values of the best fit versus the different fits for a fixed value of α. The values of β fitted by MLE (given a fixed α) were plotted and a functional form fitted to describe how β changes with temperature for the larval and adult stages (Figure 4.7; the functional forms are given in Table 4.2). In order to ensure that the average life expectancy of larvae and adults remains $1/\beta_L$ and $1/\beta_A$ respectively, the rate of progression between sub-stages was set to $\alpha_L\mu_L$ for larvae and $\alpha_A\mu_A$ for adults, where $\mu_L = 1/\alpha_L\beta_L$ and $\mu_A = 1/\alpha_A\beta_A$. This allowed modelling of gamma-distributed survival while maintaining a fixed mean life expectancy depending on temperature only.

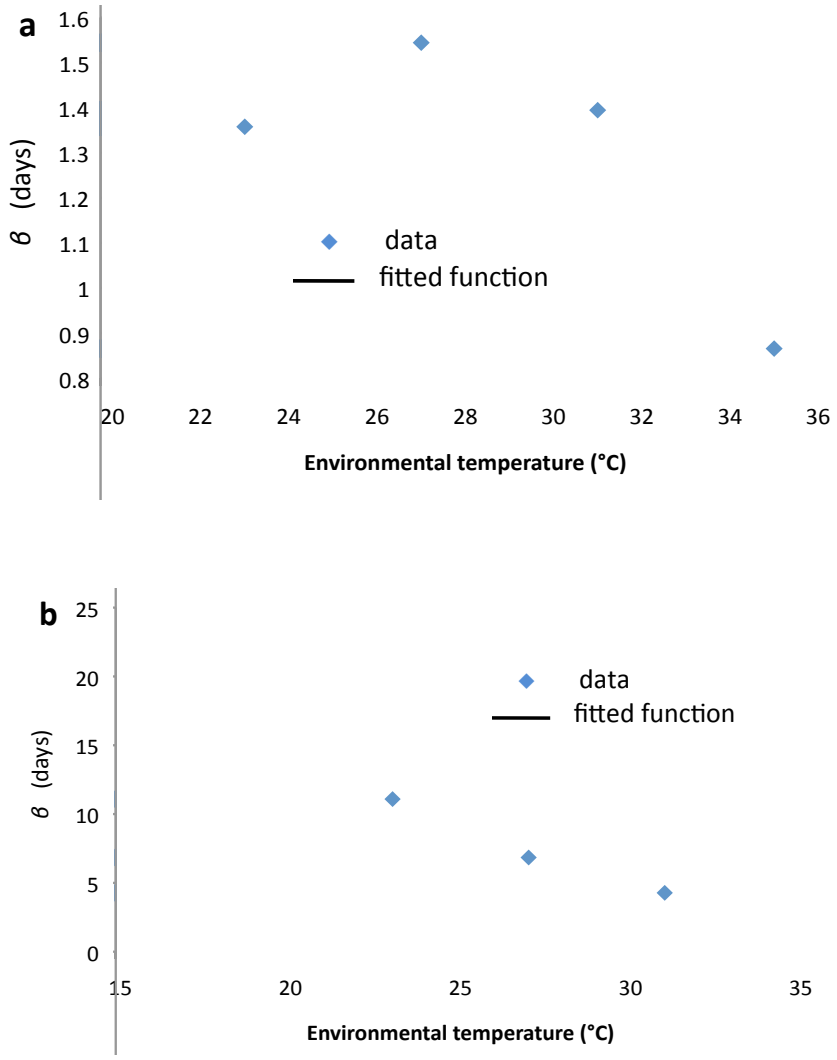


Figure 4.7. Parameter values of the gamma distribution. Value of β for the gamma larval (**a**) and adult (**b**) hazard rate as a function of environmental temperature.

Table 4.3. Values of α and corresponding fits to larval mortality data. Comparison of the MLE-defined best-fit gamma distribution to the larval mortality data at fixed values of α. The fixed value fit closest to the best fit is indicated by *.

Temperature	Value of α		Difference in AIC between Best-Fit and Fixed-Alpha Value
23 °C	MLE-determined	7.27	–
	Fixed	5	22.06
		6	6.06
		7 *	0.24
		8	1.62
		9	8.32
		10	19.08
		11	33.06
		12	49.61
27 °C	MLE-determined	5.64	–
	Fixed	5	1.46
		6 *	0.41
		7	5.17
		8	13.95
		9	25.65
		10	39.57
		11	55.2
		12	72.19
31 °C	MLE-determined	7.11	–
	Fixed	5	18.09
		6	4.4
		7 *	0.04
		8	2.25
		9	9.32
		10	20.08
		11	33.74
		12	49.74
35 °C	MLE-determined	11.11	–
	Fixed	5	210.29
		6	130.67
		7	76.24
		8	39.81
		9	16.86
		10	4.32
		11 *	0.04
		12	2.5

Table 4.4. Values of α and corresponding fits to adult mortality data Comparison of the MLE-defined best fit gamma distribution to adult mortality data, with the fits given a fixed value of α. The fixed-value fit closest to the best fit is indicated by *.

Temperature	Value of α		Difference in AIC between Best Fit and Fixed-Alpha Fit
23 °C	MLE-determined	4.675	–
	Fixed	1	90.85
		2	32.05
		3	9.5
		4	1.25
		5 *	0.24
27 °C	MLE-determined	3.401	–
	Fixed	1	137.87
		2	30.42
		3 *	1.86
		4	3.33
		5	19.89
31 °C	MLE-determined	1.844	–
	Fixed	1	40.01
		2 *	0.86
		3	34.13
		4	93.67
		5	n/a

The three models that include senescence are built upon the first, baseline model, but they include age-dependent mortality as the data presented in Chapter 2 suggest that this may be a more accurate representation of the mosquito's biology than representing survival as time-independent.

2.2.1. A model of age-dependent survival in larvae only

The second model (Figure 4.8 and equation (4.5)) includes age-dependent mortality in the larval stages only. In this model, the basic structure is the same as in Model 1, except that age-dependent survival in the larval stages is modelled by subdividing the larval phase into α_L subclasses (Wearing et al. 2005; Lloyd 2001), and the rate at which the larvae progress through the α_L subclasses is set as $\alpha_L\mu_L$, where μ_L is equal to $1/\alpha_L\beta_L$. When the eggs hatch, they enter the first larval subclass (L_1), from which they experience one of three possible events: they can progress to the next subclass (L_2) at a temperature-dependent rate $7\mu_L$, they can become pupae at a temperature-dependent rate $\sigma_L(T)$, or they can die at a density-dependent rate μ_K. These compartments are not "real" biological stages, but merely a mathematical construct to represent the gamma-distributed aging time, with the terms α_L and β_L defined by the curve fitting described in section 2.2, and Tables 4.3 and 4.4. This process applied to their progression through all subclasses.

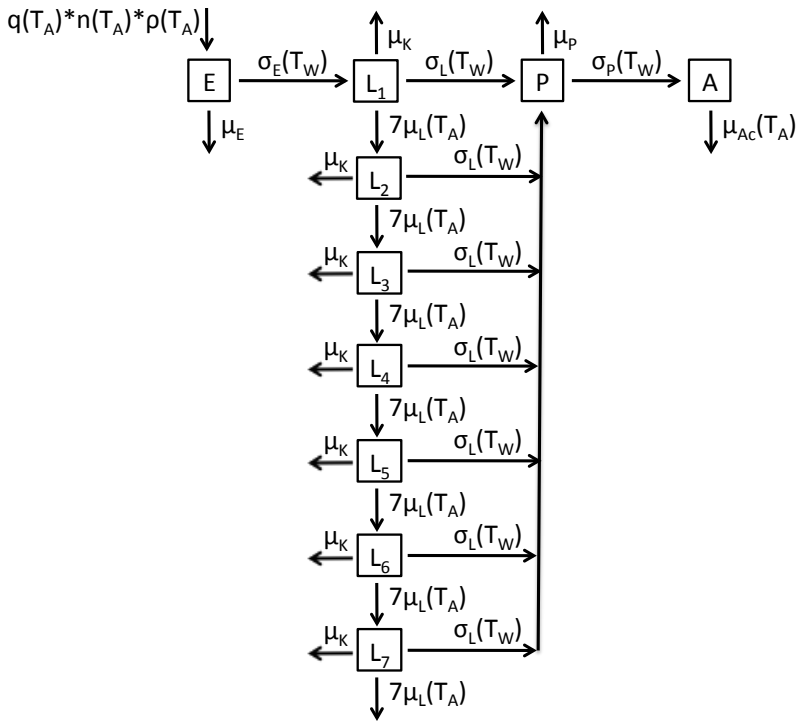


Figure 4.8. Model 2. Flow diagram representing the structure of Model 2.

$$
\begin{aligned}
\frac{dE}{dt} &= q(T_A)n(T_A)\rho(T_A)A - \left(\mu_E + \sigma_E(T_W)\right)E, \\
\frac{dL}{dt} &= \begin{cases} \sigma_E(T_W)E - \left(7\mu_L(T_A) + \mu_K + \sigma_L(T_W)\right)L_i & , \ i = 1 \\ 7\mu_L(T_A)L_{i-1} - \left(7\mu_L(T_A) + \mu_K + \sigma_L(T_W)\right)L_i & , \ 2 \le i \le 7 \end{cases} \\
\frac{dP}{dt} &= \sigma_L(T_W)\sum_{i=1}^{7} L_i - \left(\mu_P + \sigma_P(T_W)\right)P, \\
\frac{dA}{dt} &= \frac{\sigma_P(T_W)P}{2} - \mu_{Ac}(T_A)A.
\end{aligned}
\tag{4.5}
$$

2.2.2. A model of age-dependent survival in adults only

The third model (Figure 4.9 and equation (4.6)) models larval mortality as temperature- and age-independent (as in Model 1), but includes senescence as established in Chapter 2 in the form of age-dependent adult mortality. This is modelled following the same framework as the age-dependent larval mortality in Model 2, using a gamma distribution with shape parameter α_A and scale parameter β_A. The adult phase of the model comprises α_A subclasses (the number of subclasses is defined according to the curve fitting explained in section 2.2, and Tables 4.3 and 4.4), and adults move through the subclasses at the daily temperature-dependent rate

$\alpha_A\mu_A$, where μ_A is equal to $1/\alpha_A\beta_A$. Upon entering the first adult subclass (A_1), female mosquitoes necessarily progress through the adult subclasses A_1 to A_3 at a rate $3\mu_A$, until they die and drop out of the model. The number of eggs laid depends on the total number of adult females in all three adult subclasses.

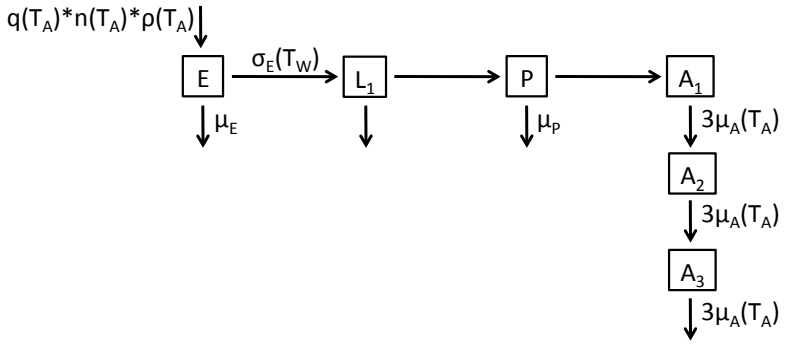


Figure 4.9. Model 3. Flow diagram representing the structure of Model 3.

$$
\begin{aligned}
\frac{dE}{dt} &= q(T_A)n(T_A)\rho(T_A)\sum_{i=1}^{3}A_i - \left(\mu_E + \sigma_E(T_W)\right)E,\\
\frac{dL}{dt} &= \sigma_E(T_W)E - \left(\mu_{Lc} + \mu_K + \sigma_L(T_W)\right)L,\\
\frac{dP}{dt} &= \sigma_L(T_W)L - \left(\mu_P + \sigma_P(T_W)\right)P,\\
\frac{dA}{dt} &= \begin{cases} \dfrac{\sigma_P(T_W)P}{2} - 3\mu_A(T_A)A_i & , \ i = 1\\ 3\mu_A(T_A)A_{i-1} - 3\mu_A(T_A)A_i & , \ i = 2,3. \end{cases}
\end{aligned}
\tag{4.6}
$$

2.2.3. A model of age-dependent survival in larvae and in adults

The fourth model (Figure 4.10 and equation (4.7)) combines the temperature- and age-dependent survival in the larval and adult stages of Models 2 and 3, and is therefore the only model to take into account fully the experimental results from Chapter 2, which indicates that both the larval and the adult stages exhibit evidence of senescence. The number of eggs produced by adult females, and the total number of pupae developing from larvae, are calculated according to the appropriate models.

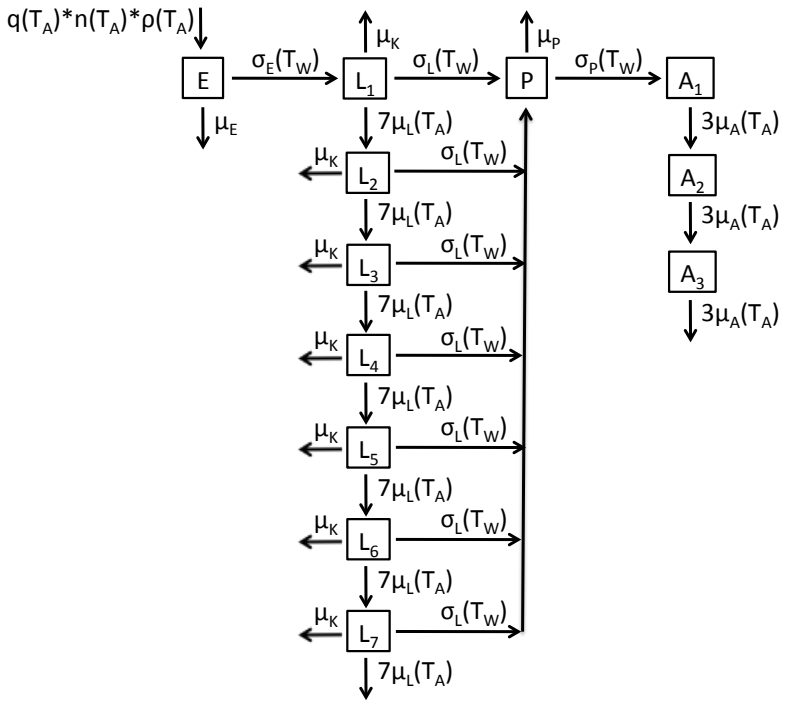


Figure 4.10. Model 4. Flow diagram representing the structure of Model 4.

$$
\begin{aligned}
\frac{dE}{dt} &= q(T_A)n(T_A)\rho(T_A)\sum_{i=1}^{3}A_i - \left(\mu_E + \sigma_E(T_W)\right)E, \\
\frac{dL}{dt} &= \begin{cases} \sigma_E(T_W)E - \left(7\mu_L(T_A) + \mu_K + \sigma_L(T_W)\right)L_i & , \ i = 1 \\ 7\mu_L(T_A)L_{i-1} - \left(7\mu_L(T_A) + \mu_K + \sigma_L(T_W)\right)L_i & , \ 2 \le i \le 7 \end{cases} \\
\frac{dP}{dt} &= \sigma_L(T_W)\sum_{i=1}^{7}L_i - \left(\mu_P + \sigma_P(T_W)\right)P, \\
\frac{dA}{dt} &= \begin{cases} \dfrac{\sigma_P(T_W)P}{2} - 3\mu_A(T_A)A_i & , \ i = 1 \\ 3\mu_A(T_A)A_{i-1} - 3\mu_A(T_A)A_i & , \ i = 2,3. \end{cases}
\end{aligned}
\tag{4.7}
$$

3. Discussion

The models developed here aim to capture the fluctuations in abundance of *An. gambiae*, and to determine whether including temperature-dependent survival, age-dependent survival, or temperature- and age-dependent survival best represent the natural population dynamics of the mosquito.

With respect to these models, there exist two main types of uncertainty: uncertainty around the parameter values and uncertainty arising from the structure of the models (Webster & Sokolov 1998; Tebaldi & Knutti 2007; Bilcke et al. 2011). The models constructed in this Chapter were elaborated as ordinary differential equations (ODEs), seeing as gamma-distributed processes (such as the survival times described in Chapter 2) are straightforward to express, both mathematically and computationally, with this type of equation (Wearing et al. 2005); other distributions of waiting times are considerably less convenient, and in some cases may be impossible, to model with ODEs. In addition, models constructed using ODEs are usually used to illustrate population-level abundance, and do not track individuals, where detailed modelling of disease vectors may call for an individual-based approach, in order to track parity, mating, and fertility, all of which affect vector abundance.

The decision to combine all four larval instars into one single stage is one example of structural uncertainty: some experimental evidence suggests that *An. gambiae* mosquitoes progress through the different larval instars at different rates (Olayemi & Ande 2009), and that some heterogeneity exists between the instars in terms of mortality, density dependence, and duration of the stages (Bayoh & Lindsay 2003; Bayoh & Lindsay 2004). Several existing models have assumed that these differences between sub-stages are significant, and have incorporated larval instars, either explicitly or implicitly, to model biological heterogeneity more realistically (Nisbet & Gurney 1983; Eckhoff 2011; White et al. 2011). The decision here not to subdivide larvae into instars was based on the absence of laboratory or field data on the effect of temperature and age on the mortality of each instar. This suggests there may be a need for further research in order better to parameterise models that include instar-specific temperature- and age-dependent survival.

It is shown here that the integer values of the gamma hazard function's shape parameter (α) that is closest to the MLE-defined value differed by temperature. This suggests that environmental temperature may affect the extent to which senescence influences mosquito mortality. In the event that temperature was significantly to affect senescence, a model with a

fixed number of larval or adult sub-stages would not be ideal, and a different model structure may be necessary.
With regards to uncertainty around model parameters, it is assumed here that eggs and pupae mortality are temperature independent, on the basis that the duration of both stages is relatively short compared with the larval and adult stages, and therefore any influence by external factors may be insignificant or comparatively minor, and will not strongly affect abundance prediction. There is currently, to my knowledge, no experimental data to indicate this assumption may be inaccurate.

Finally, the models developed here assume no interaction between the immature and mature stages, while the research presented in Chapters 2 and 3 showed that adult life-history parameters may depend on the environmental conditions during the development of the immature stages. This dependency is not taken into account in this model suite so as to focus on the role and importance of age-dependent and temperature-dependent mortality in models of *An. gambiae* population dynamics, but it may be extremely important. In addition, as was pointed out in Chapters 2 and 3, the parameterisations used here are based on four temperature data points 3°C apart for the larval phase, and three temperature data points 3°C apart for the adult phase. Fitting functional forms that hold robustly across a wider range of temperatures is clearly a limitation of this parameterisation.
Lastly, Chapter 2 shows that the Gompertz distribution generally fit the data best. The difference between the Gompertz and gamma fits is not significant for most of the temperature data points considered, and both fitted the survival data significantly better than the exponential distribution. However, the Gompertz distribution yielded the best fit overall, and therefore in order to incorporate the survival analysis as described in Chapter 2 as faithfully as possible, and thereby to represent the experimental data as realistically as possible, it may be valuable to model mortality as Gompertz-distributed, rather than gamma-distributed.

As the aim of the laboratory experiments presented in this thesis is to inform more reliably the structure and parameterisation of a model of *An.* gambiae in order to yield more accurate predictions of vector population dynamics, it is important to determine to what extent, if at all, the inclusion of age-dependent survival as suggested by the results in Chapter 2, or of temperature-dependent parameters as suggested by the results in Chapter 3, impact model performance, or whether current parameterisations of vector models are sufficient.

CHAPTER 5

MODEL FITTING AND IMPLICATIONS OF VECTOR AGE- AND TEMPERATURE-DEPENDENT SURVIVAL FOR ABUNDANCE MODELS

1. Introduction

In the context of gaining a better understanding of the role of climatic factors on vector abundance, and therefore on VBD transmission, developing reliable mathematical models is a powerful tool with which to comprehend and interpret disease trends under current and future climate scenarios. In the case of mathematical models of VBDs, the vector component of the model is particularly important seeing as the survival and development of the vectors is poikilothermic, and therefore it is important that such models are built to represent vector population dynamics and their response to climatic factors as realistically as possible.

In the case of malaria and its main vector in Africa, *An. gambiae*, it has been shown experimentally that a range of mosquito life-history parameters depend on environmental temperature, as well as other climate-related elements (Ng'habi et al. 2005; Dawes et al. 2009; Semenza & Menne 2009; Takken & Koenraadt 2013), and it is becoming more common for models of vector population dynamics to include climatic elements explicitly in their structure or parameterisation (Hoshen & Morse 2004; Depinay et al. 2004; Ermert et al. 2011; White et al. 2011; Parham et al. 2012; Beck-Johnson et al. 2013). However, this is a relatively new field of research, and to my knowledge there exists currently no experimental data showing senescence in *An. gambiae* s.s. mosquitoes, and therefore the implications of age-dependent mortality have not been widely explored in modelling studies yet, although some research, as well as the results presented in Chapter 2, suggests this may be relevant (Clements & Paterson 1981; Dawes et al. 2009; Hancock et al. 2009; Bellan 2010; Arifin et al. 2014). As the assumption that incorporating temperature- or age-dependence in mathematical models is not universally adopted, and indeed including temperature-dependent vector life-history parameters, or senescence, may yield no advantages in terms of model goodness of fit to data, further investigation is needed into the effect of including this additional complexity in vector models. An argument could be made, if the data support it and

show little improvement in model fitting and predictions, to model survival and other parameters as temperature- and age-independent for the sake of model parsimony.

Chapter 2 provides clear evidence that senescence occurs in *An. gambiae* and is temperature-dependent, and Chapter 3 defines key reproductive parameters such as the proportion of females laying eggs, the number of eggs a female mosquito can lay, and the proportion of eggs that hatch: this provides an insight into the life-history that has previously not been taken into account when designing mathematical models of mosquito population dynamics.

The suite of four models of *An. gambiae* s.s. described in Chapter 4 was developed to test whether including these experimental results, namely temperature- and age-dependent survival, as observed in the laboratory, in a model of mosquito abundance may fit surveillance data from sub-Saharan Africa better than assuming a fixed environmental temperature or the standard modelling paradigm of age-independent mortality rates. This chapter compares the performance of models that include age- and/or temperature-dependent survival to that of a 'standard' baseline model that models survival as time-independent: this goes beyond existing literature both in terms of the inclusion of senescence in mathematical models of *Anopheles* abundance (as per the models developed in Chapter 4, based on the experimental data in Chapters 2 and 3), and in terms of comparing the goodness of fit of these novel models to that of a model that does not include senescence, and that is therefore representative of the assumptions made in current research with regard to survival. In order to quantify the extent to which including temperature-dependent and age-dependent mortality influences model output, the four models developed in Chapter 4 were fitted here to the longitudinal datasets of adult *An. gambiae* abundance in sub-Saharan Africa.

2. Methods

2.1. Longitudinal data for model fitting

A systematic literature review was carried out across the databases PubMed, Web of Knowledge, Google Scholar, and Ovid SP, and all articles published up until February 14, 2015 were considered. Primary key terms *Anopheles*, *gambiae*, *Africa*, *long-term*, *longitudinal*, and *temporal*, and secondary terms *abundance*, *abundance data*, *population*, *density*, *population density*, *mosquitoes*, *monthly*, *weekly*, and *daily* were used in combination with Boolean operators to direct searches. The titles and abstracts of resulting searches were screened for their likelihood to contain data on the collection of *An. gambiae* mosquitoes in any African country over a minimum continuous period of 12 months.

If the initial requirements were met, the full article was retrieved and detailed inclusion and exclusion criteria applied. *An. gambiae* s.s. was the primary focus of data collation, but articles with data on *An. gambiae* sensu lato were also accepted. Records of abundance data collected monthly, weekly, or daily were included provided studies met the requirement of a minimum collection period of 12 months between 1st January 2000 and 31st December 2009. Studies that reported only annual abundance data were excluded. Abundance data for all life-history stages (eggs, larvae, pupae, or adults) were accepted, although the majority of articles reported adult mosquito counts exclusively. Only studies reporting on the natural population dynamics of *An. gambiae* in the wild were included: abundance data collected during implementation of vector control intervention programmes, or from studies that chemically or genetically modified mosquito populations were excluded. Household collection data were accepted only if abundance data were from outdoor collections, and available for all households within the geographical region of the study. The bibliography list of articles meeting all criteria was also examined for additional relevant references.

Where raw data were available, mosquito numbers were lifted directly from the article, while data presented only in figures were extracted using GraphClick, Version 3.0.2 (Arizona Software, 2010). For articles in which abundance data were mentioned (or referred to) but not explicitly given, or where the data could not be recorded, the study authors were contacted directly to request the raw data. When authors did not respond, or if the data did not meet the inclusion criteria, the article was excluded. Where geographical coordinates were explicitly given, the city and country were entered into (Zwiefelhofer 2015) to retrieve latitude and longitude. Locations were mapped using BatchGeo (BatchGeo LLC 2015).

A summary of the systematic literature review process and results is shown in Figure 5.1. The review unearthed 12 datasets within 10 publications that fulfilled all inclusion criteria. For each of these datasets, the following information was recorded: title; authors; city, country, and geographical coordinates of the study site; start and end date of the study; study duration in months; and the mosquito species and life-history stage that was counted (Table 5.1). The mapped locations of the villages or areas across sub-Saharan Africa where the abundance data were recorded are shown in Figure 5.2.

The systematic review indicates that while longitudinal *An. gambiae* abundance data exist for several locations across sub-Saharan Africa at different climates, altitudes, and within different environments, these vary widely in terms of duration, ease of availability, provision of sufficient detail to enable robust model fitting, and overall data quality. Since the main purpose of this review was to assemble a collection of datasets that could be used to inform

model fitting, it is clear that longer time-series would enable better model fitting to temporal trends in the data. Only half the datasets obtained described monthly-resolution mosquito abundance for a period longer than 2 years (which would facilitate consistent vector behaviour and climate-driven population response to emerge as distinct temporal patterns, and thus would increase the likelihood of a good fit to the data (Churcher et al. 2015)).

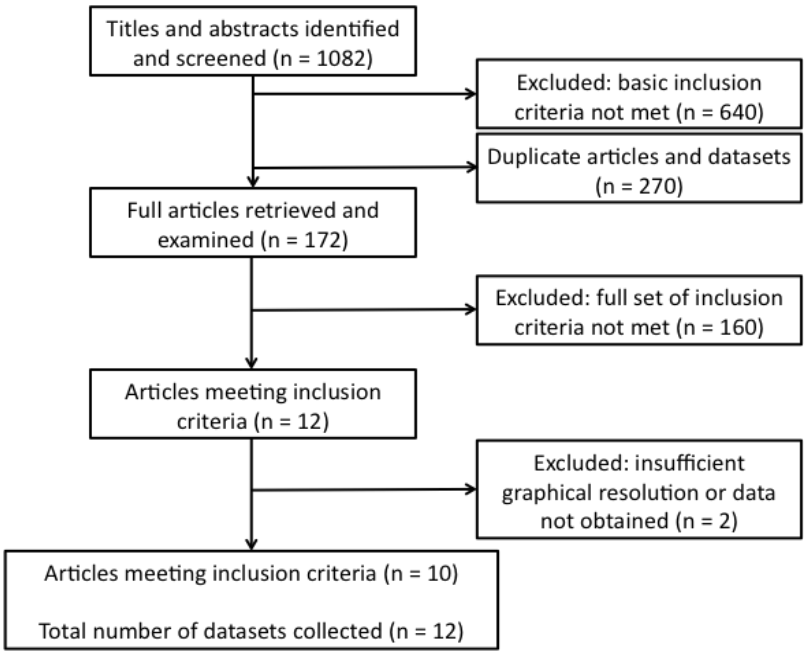


Figure 5.1. Systematic literature review flowchart. This describes the literature review process and results, and the number of articles and abundance datasets collected.



Figure 5.2. Map of geographical locations of datasets. The regions of sub-Saharan Africa where the abundance datasets described in Fig. 5.1 and Table 5.1 were collected.

Table 5.1. Longitudinal datasets of *An. gambiae* abundance. These are the 12 datasets that matched the inclusion criteria as described in section 3.1 here, and in Figure 5.3.

Map ref	Geographical location	Study dates	Duration (months)	Mosquito species and stage
A	Likoko, Cameroon (Tanga et al. 2011)	Oct 2002 – Sep 2003	12	*An. gambiae* s.l. adults
B	Mutengene, Cameroon (Tanga et al. 2011)	Oct 2004 – Sep 2005	12	*An. gambiae* s.s. adults
C	Ekombitié, Cameroon (Fils et al. 2010)	Jan 2007 – Dec 2007	12	*An. gambiae* s.l. adults
D	Njabakunda, The Gambia (Nwakanma et al. 2013)	Apr 2007 – Mar 2009	24	*An. gambiae* s.s. adults
E	Kassena, Ghana (Kasasa et al. 2013)	Nov 2001 – Oct 2004	36	*An. gambiae* s.s. adults
F	Kintampo, Ghana (Dery et al. 2010)	Nov 2003 – Nov 2005	25	*An. gambiae* s.s. adults
G	Banizoumbou, Niger (Gianotti et al. 2008)	May 2005 – Dec 2006	20	*An. gambiae* s.l. adults
H	Zindarou, Niger (Bomblies et al. 2009)	July 2005 – Dec 2006	18	*An. gambiae* s.l. adults
I	Ogbakiri, Nigeria (Uttah et al. 2013)	Sep 2005 – Aug 2006	12	*An. gambiae* s.l. adults
J	Fort Ternan, Kenya (Imbahale et al. 2011)	Mar 2006 – Mar 2008	25	*An. gambiae* s.l. larvae
	Lunyerere, Kenya (Imbahale et al. 2011)			
	Nyalenda, Kenya (Imbahale et al. 2011)			
K	Masaïka, Tanzania (Meyrowitsch et al. 2011)	Jul 1998 – Nov 2001	41	*An. gambiae* s.l.

The quality of these datasets, and the level of detail with which abundance data are recorded and/or made available, strongly influenced the ability to fit the four models to the datasets, and, hence, the accuracy of the models' predictions. Although 12 datasets matched the inclusion criteria, the methods sections in their respective publications almost always reported only the number of mosquitoes caught per month, with very little (or no) information on the number of catches or the frequency of catches within a month. This unavoidably results in model fitting to these datasets requiring *a priori* assumptions in the likelihood expression about the mean and standard deviation around the total monthly number of catches.

Here, since the strength (and likely effect) of these assumptions increases for datasets reporting shorter time-series, the four models developed in Chapter 4 (summarised in Table 5.2) were fitted only to the two longest datasets: one spanning 36 months in Ghana (Kasasa et al. 2013) and the other 41 months in Masaïka, Tanzania (Meyrowitsch et al. 2011). These two datasets were chosen because they comprise monthly-resolution adult mosquito abundance for a period of at least 3 years, and it is expected that longer time-series would allow vector behaviour to emerge as a consistent pattern, and would therefore enable better model fitting to temporal trends in the data.

Table 5.2. **Recapitulation of the models developed in Chapter 4**. This table recalls the main distinguishing features of the four models constructed in Chapter 4, namely the inclusion or not of age-dependent survival in the larval and/or adult stages.

Model	Larval age-dependent survival	Adult age-dependent survival	Reference section in Chapter 4
1	No	No	2.1, Fig. 4.3, Equation 4.3
2	Yes	No	2.2.1, Fig. 4.8, Equation 4.5
3	No	Yes	2.2.2, Fig. 4.9, Equation 4.6
4	Yes	Yes	2.2.3, Fig. 4.10, Equation 4.7

The climate data needed to run the models' fit to the data gathered in Kassena, Ghana (10° 30' 0" N, 1° 0' 0" W), namely daily average temperature values (t2m, defined as the average temperature two metres above the ground over a 24-hour period, Figure 5.3A) and daily precipitation values (defined as the average rainfall for a 24-hour period, Figure 5.4A) for the period spanning November 1st, 2001 until October 31st, 2004 to match the abundance data, were provided from the ECHAM5/MESSy2 Atmospheric Chemistry (EMAC) general circulation model for the nearest geographical point to Kassena at a resolution of 50 kilometres, with the closest point to Kassena at 10° 37' 30" N, 1° 7' 30" W (Dr. Yannis Proestos, pers. communication) (Roeckner et al. 2003; Jöckel et al. 2006; Proestos et al. 2015). The climatic boundaries for the model were set using AMIP-II (Taylor et al. 2000) sea-surface

temperature (sst) and sea-ice coverage (sic) assimilation data (Dr. Kamil Ergüler, pers. communication).

The climate data used to run the models and allow fitting to the data from Masaïka, Tanzania (5° 16' 0" S, 38° 49' 60" E) were extracted from the ECMWF ERA-40 re-analysis dataset (European Centre for Medium-Range Weather Forecasts 2014), whose nearest point to the community of Masaïka was located at 5° 0' 0" S, 37° 30' 0" E. This dataset provided daily temperature values (given as the average of a 24-hour period, shown in Figure 5.3B) and daily rainfall values (given as the cumulative amount of rainfall measured at 6pm every day, Figure 5.4B) for the period spanning July 6th, 1998, until November 30th, 2001, to match the dates for which abundance data was available (Dr. Wes Hinsley, personal communication).

In order to test the hypothesis that modelling certain mosquito life-history parameters as temperature-dependent does not result in more accurate predictions of population dynamics, the four models described above were fitted to each dataset twice: once with the fluctuating environmental temperatures determined by the datasets described above, and once with a fixed environmental temperature of 26°C. The fixed temperature was chosen to be 26°C because the mean of the ERA-40-determined daily temperature values matching the Ghanaian abundance dataset was 26.2°C (Figure 5.3A) and the mean of the EMAC-determined daily temperature values for the Tanzanian abundance dataset was 26.4°C (Figure 5.3B).

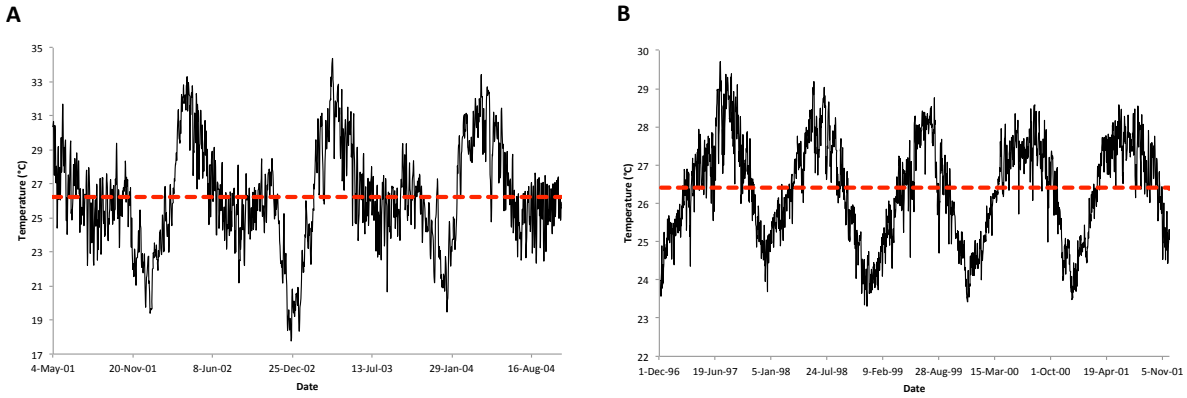


Figure 5.3. Daily temperature profiles. This black line shows the daily temperature values for Kassena, Ghana (A) from May 4th 2001 until October 31st 2004, and for Masaïka, Tanzania (B) from December 6th 1998 until November 30th 2001. The red line shows the overall average temperature for both datasets: 26.2°C in Ghana, and 26.4°C in Tanzania.

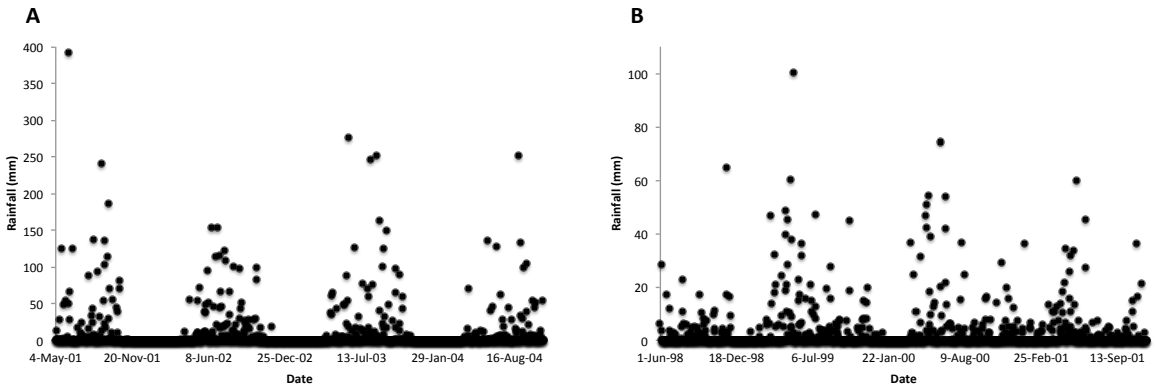


Figure 5.4. Daily rainfall profiles. The points represent the daily rainfall in mm in Kassena, Ghana (A) for the period between May 4th 2001 and October 31st 2004, and in Masaïka, Tanzania (B) for the period between June 1st 1998 and November 30th 2001.

2.2. Model fitting

The performance of the models developed in Chapter 4 was tested by fitting to existing *An. gambiae* s.s. surveillance data and comparing model fits. The fitting was carried out using the hoppMCMC (version 0.4) algorithm (Ergüler 2015) for Python (version 2.7.6), using the default parameters, which performed the fitting of the parameters described in Table 5.3 by minimising the score function, given as

$$S = \frac{1}{2}\sum_t \left(\frac{\delta_t - \gamma_t}{\sigma} \right)^2, \qquad (1)$$

where δ_t is an observation at time point t, y_t is the simulated point corresponding to the observation at time point t, and σ is the standard deviation around the observed data. A simulated point corresponds to the sum of adult mosquitoes simulated each day during the month covered by the observation. Every data point represents a monthly average of the number of mosquitoes caught over a number of days throughout each month: as the data points were lifted from figures in the papers, there was no information as to standard deviations around each monthly average, so it was assumed that the standard deviation for each observation is the maximum value of all the observations for the dataset from which the observation was taken.

The hoppMCMC algorithm was chosen as it is tailored to address a range of fitting problems and is designed to sample from the posterior distribution (allowing the estimation of the 95% range of the parameters it is fitting) as well as to find the best-fitting parameter values: this provided a more comprehensive approach to model fitting and parameter estimation than Python's widely used basin-hopping algorithm, which only determines the best-fit parameter values without allowing for analyses around the parameter values. While it is possible to perform sensitivity analyses separately on the best-

fit parameters determined by basin-hopping, the fact that the hoppMCMC algorithm performs both the model fit and the posterior distribution sampling minimises compatibility issues and allow for immediate interpretation of the best-fit parameter values.

The models were implemented and run in C, and integrated using the LSODA solver in the SciPy package for Python (v. 2.7.6).

Table 5.3. Parameters inferred by fitting. This table is an excerpt from Table 4.2 in Chapter 4, recalling the parameters that were inferred during the process of model fitting, and their prior values. The references for the prior values are given in Table 4.2.

Parameter	Definition	Unit	Prior value
q	Proportion of adult females laying eggs	–	0.61-0.85
μ_E	Per capita egg mortality rate	$days^{-1}$	0.32-0.8
μ_C	Constant	$days^{-1}$	1-10000
τ	Days of rainfall contributing to carrying capacity	days	<10
ΔT	Difference between air and water temperature§	°C	2.9-7.6

The Pearson correlation coefficient, r, was calculated on the log of the data as the data are not normally distributed. The coefficient was calculated using the results of the simulation with the best-fit parameter set, in order to define the strength of the relationship between the data points and the model output. In addition to this, the AIC was calculated to facilitate comparison between models:

$$AIC = 2k - 2\ln L, \quad (2)$$

where k is the number of parameters, and $\ln L$ is the logarithm of the likelihood (Bolker 2008), which is proportional to the score function (Zivot 2009):

$$\ln L = -S - n\ln\sqrt{2\pi} - \ln\prod_t \sigma. \quad (3)$$

In order to test the models' sensitivity to the inferred parameter values, the marginal 95% range (2.5-97.5%) of each parameter was obtained from the posterior samples. This 95% interval indicates the range within which a parameter's value generates a similar model fit and results in a similar model score.

3. Results

3.1. Model fitting

Figure 5.5 and 5.6 compare the performances of Models 1 to 4 with the abundance data reported in Meyrowitsch et al. (2011) and Kasasa et al. (2013) respectively. Table 5.4 gives the AIC values for each model and Pearson's correlation coefficients (r) for the correlation between each model and the logs of the two datasets.

The correlation coefficients shown in Table 5.4 suggest that for Models 1 and 2, artificially fixing the environmental temperature to 26°C results in simulations that are not significantly different from when the models were fitted to the variable daily temperatures, although the fit to the data from Tanzania was very poor. Models 3 and 4 also appeared to yield a considerably better fit with fixed than varying temperature when fitted to the Tanzanian data, but not when fitted to the Ghanaian data: in this case, fitting the models to fluctuating temperatures resulted in considerably more faithful simulations than fitting to fixed temperature. While it is commonly accepted that a difference of ≤ 2 in AIC values suggests models are more or less indistinguishable, information criteria do not provide a measure of statistical significance: models with a lower AIC fit the data better, but this does not give an indication of how much better they fit (Kass & Raftery 1995; Bolker 2008).

The fitted population dynamics and performance of Models 1 and 2 are very similar, and Models 3 and 4 are almost identical. The models incorporating adult-only senescence (Model 3) and adult and larval senescence (Model 4) fit the data slightly better than the model with no senescence in any stage (Model 1) and with larval-only senescence (Model 2). While the difference in AIC values between Models 1 and 2, and Models 3 and 4, is less than 2, the correlation coefficients show that Models 3 and 4 fit the data considerably better than Models 1 and 2, and this is consistent with the marginal 95% range shown in Figures 5.5 and 5.6.

It is clear from both figures that the fit of Model 1 is considerably worse than all other models at reliably capturing the magnitude of the main data peaks and troughs, as the points describing the peaks and troughs generally lie outside the 95% range. In addition, Model 1 appears to predict a forward shift in the simulated trajectory compared with the surveillance data from Tanzania, where it fails to predict not only the magnitude but also the timing of the peaks and troughs. Model 2 captures some of the troughs better than the first model, but the 95% ranges still fail to include the extreme peaks in mosquito abundance, and fitting to the dataset from Tanzania also resulted in a forward shift, as was the case for Model 1. The fits of Models 3 and 4 are both much closer to both datasets, and appear to capture particularly well the surveillance data from Ghana (Figure 5.6, C), and the 95% ranges of both

models generally capture well both the peaks and troughs in the time-series. Both Models 3 and 4 appears to simulate the abundance data from Tanzania with a very slight forward shift (Figure 5.5, C and D respectively), and to result in a broader peak in mosquito abundance, but the Pearson correlation coefficients indicate these models still fit the data considerably better than the first two models, which do not include adult age-dependent survival.

It is evident from the AIC values and correlation coefficients that Models 2 and 4, which include age-dependent mortality in the larval stage, yield no improvement on the fitting compared, respectively, with Models 1 (temperature-dependent mortality only) and 3 (age-dependent mortality in the adult stages only).

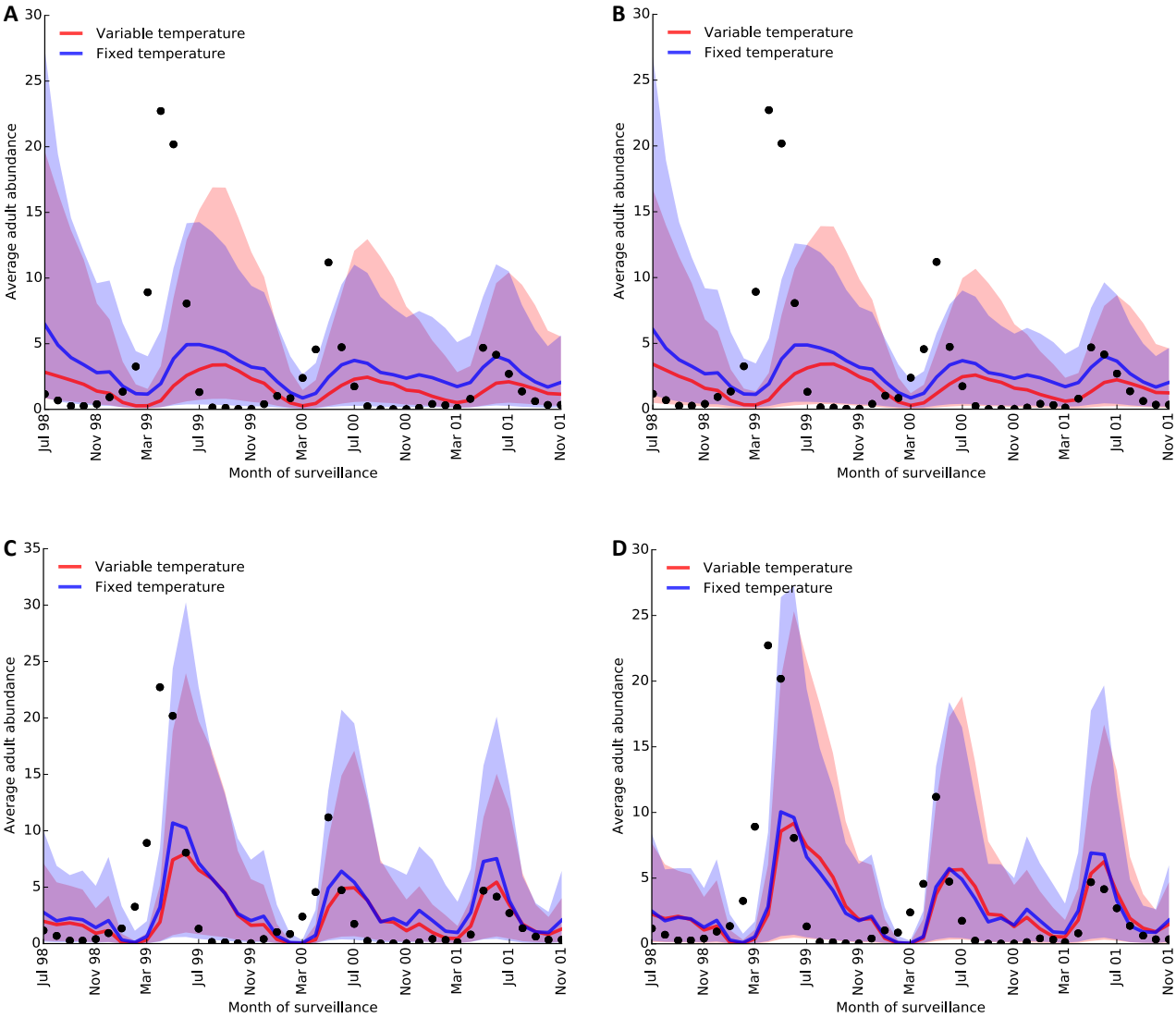


Figure 5.5. Model fitting to abundance data from Tanzania (Meyrowitsch et al. 2011). Simulation results of models 1 (A), 2 (B), 3 (C), and 4 (D) run with fixed temperature (T=26°C, blue lines) and variable temperature (red lines) plotted against the surveillance data (black points). Solid lines show the simulated abundance predicted by the best-fit parameter set. Shaded areas cover 95% of the model's output when simulations are performed with posterior parameter samples.

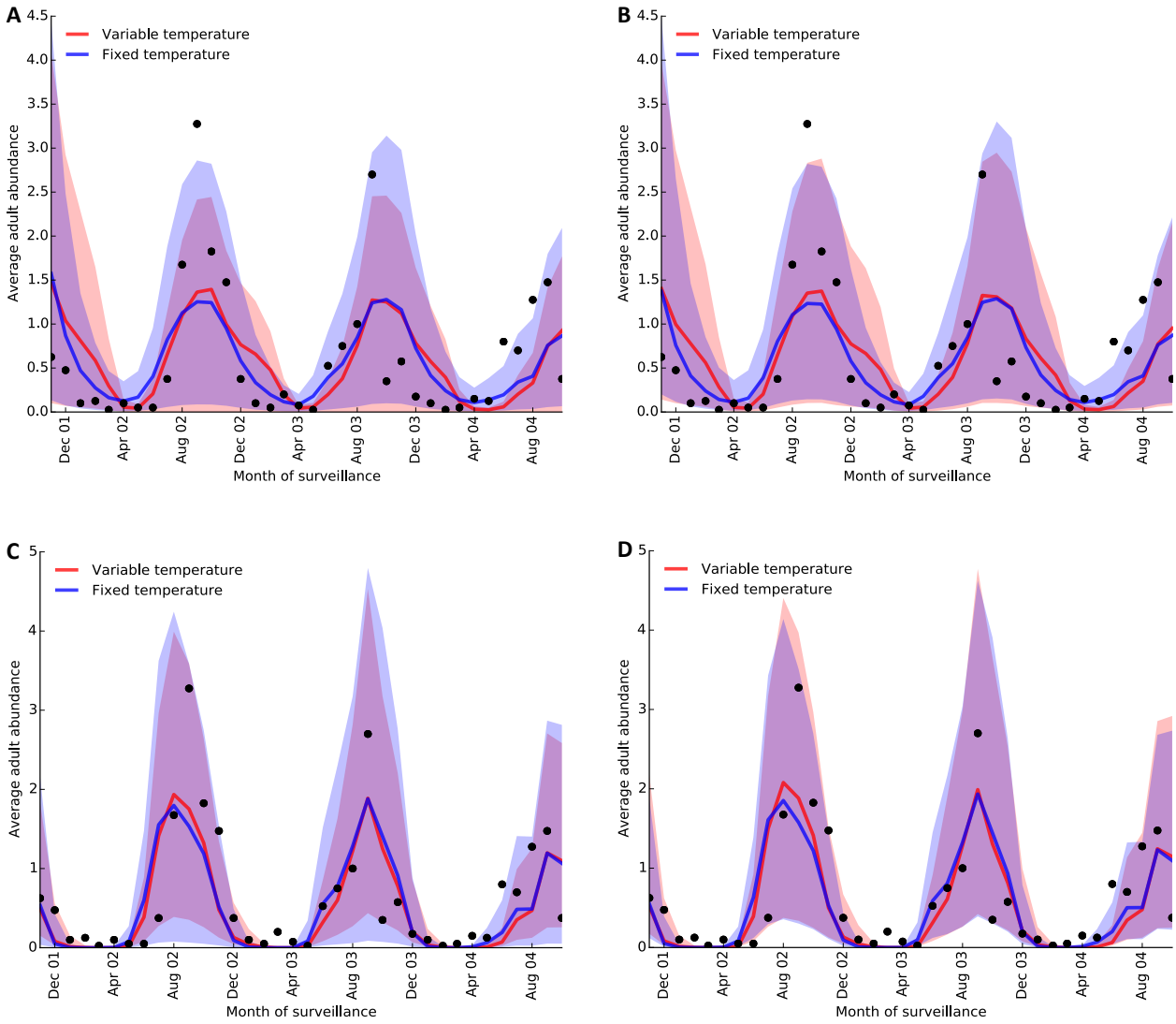


Figure 5.6. Model fitting to abundance data from Ghana (Kasasa et al. 2013). Simulation results of models 1 (A), 2 (B), 3 (C), and 4 (D) run with fixed temperature (T=26°C, blue lines) and variable temperature (red lines) plotted against the surveillance data (black points). Solid lines show the simulated abundance predicted by the best-fit parameter set. Shaded areas are the marginal 95% range.

Table 5.4. Model Statistics. The AIC for each model, and the Pearson correlation coefficients (*r*) of the log of the data, describing the quality of the models' fit to the datasets (Dataset 1 is Meyrowitsch et al. (2011), Dataset 2 is Kasasa et al. (2013)). Var T corresponds to variable temperature, and fix T to fixed temperature.

Dataset	Fit	Model 1		Model 2		Model 3		Model 4	
		var T	fix T	var T	fix T	var T	fix T	var T	fix T
1	AIC	356	355.86	356.01	355.85	355.50	355.32	355.50	355.30
	r	0.24	0.12	0.32	0.09	0.66	0.59	0.69	0.63
2	AIC	174.36	174.26	174.35	174.22	173.89	174.02	173.88	174.01
	r	0.74	0.75	0.76	0.78	0.82	0.75	0.83	0.76

3.2. Best-fit parameter values

Table 5.5 shows the best-fit parameter values for each model when fitted to each dataset. All four models were consistent in their predictions of very similar proportions of adult females that lay eggs (q), and of daily egg mortality rates (μ_E). For both these parameters, the best-fit values fell well within the prior ranges assumed based on existing experimental data (as shown in Chapter 4, Table 4.2, based on (Okogun 2005; Lutambi et al. 2013)) and the results presented in Chapter 3.

The models, including the two best fitting models (3 and 4), generated predictions for quite a wide range of values for the difference between environmental air and water temperature. Fitting to the dataset from Tanzania (Meyrowitsch et al. 2011), the average value of ΔT predicted across all models, was 4.32°C (±1.25°C standard deviation) when taking into account temperature fluctuations, and 5.428°C (±1.669°C) when assuming a constant environmental temperature of 26°C; when fitting to the Ghanaian dataset (Kasasa et al. 2013), the average value of ΔT was 6.566°C (±0.777°C) with temperature fluctuations, and 5.455°C (±1.465°C) with fixed temperature. These values are in the middle of the range observed in experimental data, where the difference between air temperature and water temperature was found to depend on the size of the water pool, indicating that the model predictions here are in all line with the difference in air and water temperature observed for medium-sized pools (K. P. Paaijmans et al. 2008). These results suggests that models assuming no difference between air and water temperature may not realistically capture the full effect of temperature on survival, likely leading to an underestimate of the influence of temperature on the mortality of the aquatic stages: as it was shown in Chapter 2, larvae exposed to air temperatures of 35°C do not survive to adulthood. However, given that water temperature is likely to be at least a couple of degrees higher than air temperature, it can be assumed that the minimum temperature at which larvae cannot develop is, in fact, lower.

Models 3 and 4 predict very small values for the weighting factor on the number of rainfall days τ contributing to breeding site carrying capacity (and therefore to density-dependent mortality of the larval stages) across both datasets. As this value corresponds to less than one day, this indicates that for both models for these particular datasets, the cumulative effects of rainfall are weighted almost equally. Model 1 predicts values of τ larger than 1 when fitted to the Tanzanian dataset, and Model 2 predicts values of τ larger than 1 for both datasets, indicating that for these models, recent rainfall is more important in determining the carrying capacity of breeding pools.

Table 5.5. Posterior parameter values. Best-fit parameter values for each model fitted to both datasets. Parameters are described in Table 5.2, and parameter prior values are taken from Chapter 4, Table 4.2. V stands for Variable Temperature, F for Fixed Temperature.

Dataset	Models		Parameters				
			q	μ_E	μ_C	τ	ΔT
Prior values			**0.61-0.85**	**0.32-0.8**	**0-10000**	**<10**	**2.9-7.6**
Meyrowitsch et al. 2011	Model 1	V	0.801	0.452	2713.07	1.846	5.974
		F	0.632	0.344	1083.77	3.462	6.401
	Model 2	V	0.779	0.544	5201.48	4.061	3.081
		F	0.785	0.615	775.04	3.354	5.948
	Model 3	V	0.663	0.661	8090.32	0.751	4.497
		F	0.800	0.470	9037.13	0.812	2.945
	Model 4	V	0.753	0.561	3126.17	0.726	3.719
		F	0.797	0.796	9714.09	0.484	6.416
Kasasa et al. 2013	Model 1	V	0.611	0.343	3099.46	0.968	5.680
		F	0.654	0.617	3712.47	0.911	6.569
	Model 2	V	0.619	0.676	1771.86	1.512	7.067
		F	0.686	0.645	1976.22	1.029	3.653
	Model 3	V	0.688	0.603	6703.26	0.449	6.167
		F	0.683	0.678	6313.26	0.748	4.873
	Model 4	V	0.661	0.645	9163.71	0.306	7.350
		F	0.807	0.699	7203.96	0.735	6.726

3.3. Marginal 95% range of posterior parameter values

The marginal 95% range of each of the five fitted parameters (Table 5.6) indicates that the proportion of adult females laying eggs (q), as well as, to a slightly lesser extent, the daily mortality rate of eggs (μ_E) only generate similar model predictions for a very narrow range of parameters. This suggests that model outputs are likely to be particular sensitive to changes in these parameters, as any parameter value outside the narrow 95% range will result in a different fit. This is to be expected, as these parameters are crucial in the reproduction, fitness, and survival of the mosquito, and serves to highlight on which entomological parameters we need more detailed and precise data in order to produce more reliable and robust models of disease vector population dynamics. The difference between environmental air and water temperatures (ΔT) also has a relatively narrow 95% range, which further emphasises the importance of modelling the higher temperatures of the water bodies in which immature stages of the mosquito develop, and suggests that models assuming water temperatures are

the same as air temperatures may be missing out on a subtle determinant of mortality. This also highlights the necessity of measuring water temperature specifically in experimental work that aims to define the effect of temperature on the survival of *An. gambiae* aquatic stages, although this observation is likely applicable to other mosquito disease vectors also. The small 95% range for the parameter describing the difference between air and water temperature also emphasises that the population dynamics of *An. gambiae* are dependent not just on mean environmental temperatures, but also on small temperature fluctuations, which is consistent with previous experimental and theoretical work (Beck-Johnson et al. 2013; Lyons et al. 2013).

Table 5.6. The marginal 95% range (2.5-97.5%) of each parameter. This was obtained from the posterior samples for each model fitted to both datasets, and allowed an analysis of the sensitivity of the model to changes in parameter values. V indicates variable temperature, F indicates fixed temperature.

	Models		**Parameters**				
			q	μ_E	μ_C	τ	ΔT
Meyrowitsch et al. 2011	Model 1	V	0.61-0.85	0.33-0.78	2145.2-9776.9	0.29-9.46	3.01-7.34
		F	0.62-0.84	0.34-0.78	1789.7-9770.9	0.50-9.60	3.21-7.37
	Model 2	V	0.62-0.84	0.37-0.79	2661.1-9703.4	0.25-9.48	2.95-7.36
		F	0.61-0.84	0.35-0.79	2004.6-9814.0	0.55-9.24	3.25-7.40
	Model 3	V	0.62-0.83	0.34-0.79	1964.9-9856.9	0.33-9.35	3.18-7.44
		F	0.62-0.84	0.33-0.79	2086.9-9813.0	0.28-9.80	3.14-7.44
	Model 4	V	0.62-0.84	0.35-0.78	2244.9-9774.8	0.33-9.73	3.11-7.51
		F	0.62-0.84	0.35-0.79	2165.8-9866.2	0.30-9.72	3.13-7.45
Kasasa et al. 2013	Model 1	V	0.61-0.84	0.34-0.78	1147.8-9674.1	<0.1-8.81	2.99-7.14
		F	0.62-0.83	0.34-0.79	3127.9-9873.1	0.72-9.42	2.98-7.13
	Model 2	V	0.62-0.84	0.35-0.79	2010.7-9737.3	0.71-9.85	3.05-7.34
		F	0.62-0.83	0.33-0.79	2177.4-9770.7	0.73-9.66	3.19-7.42
	Model 3	V	0.62-0.84	0.33-0.78	2181.6-9926.7	0.32-9.62	2.97-7.52
		F	0.61-0.84	0.33-0.78	1994.8-9842.8	0.37-9.70	3.05-7.48
	Model 4	V	0.61-0.84	0.34-0.79	2071.1-9754.4	0.28-9.39	2.96-7.40
		F	0.61-0.84	0.33-0.78	1487.1-9800.8	0.31-9.65	3.0-7.40

4. Discussion

According to the fits to data of the models developed in Chapter 4, including temperature-dependence in several parameters of models of *An. gambiae* s.s. abundance may not yield much benefit compared

with temperature-independent parameters. However, this result may be due to the manner in which temperature-dependence was included in the parameters here: it is possible that these models cannot respond to fluctuating temperatures as flexibly as would be required to capture the benefit of including daily temperature changes. Further investigation is needed to determine whether this is a property of these particular models, or whether modelling temperature-dependent parameters may indeed be minimally advantageous.

On the other hand, incorporating age-dependent survival in the adult stages of models of An. gambiae s.s. population dynamics may provide significantly better fits to longitudinal adult female abundance data from sub-Saharan Africa. Despite the limitations and assumptions of this study (discussed further down), it is shown that models incorporating age-dependent mortality in the adult stages match temporal trends in observed mosquito data better than models without age-dependent mortality or models that include age-dependent mortality in the larval stages only. This suggests that incorporating age-dependent mortality in the larval stages merely serves to increase the complexity and dimensionality of models, but without contributing to overall model performance or predictive ability. Given the desire, wherever possible, for parsimony in model construction, including age-dependent survival in the adult stages when modelling *An. gambiae* population dynamics appears to be an important requirement for developing more realistic models, whereas including age-dependent survival in the larval stages does not improve model predictions. Given this result, and the fact that age-dependent mortality has been reported in *Aedes aegypti* (Harrington et al. 2008), the common assumption of time-independent adult *An. gambiae* mortality (or even modified to incorporate temperature dependence), or, equivalently, that senescence does not play a large role in driving vector mortality, may be erroneous or, at the very least, a serious underestimation, and it may prove equally important to include senescence in models of other mosquito population dynamics and mosquito-borne diseases. However, this result requires further research and confirmation, both from more detailed vector mortality data collected the field, additional experimental data from the laboratory (e.g. on other mosquito species), and further modelling studies of both the mosquito populations themselves and the diseases they transmit.

The models aim to capture the population dynamics of *An. gambiae* in the wild, but it should be noted that the calibration of parameters is based on data obtained on laboratory mosquitoes (essentially due to the scarcity of data available on wild mosquito life history parameters). It may be that mosquitoes in the laboratory are not, in fact, truly representative of wild populations, in which case the parameters adopted here may be limited in their application to modelling *An. gambiae* population dynamics in sub-Saharan Africa, and the fit of these models to datasets used should be interpreted with caution. This highlights again the need for better entomological experimental data to inform models. In

addition, the parameterisation adopted is time-independent, based on the assumption that there was no phenotypic change between mosquitoes collected in abundance datasets from different periods. However, recent research has suggested that insect vectors can evolve over decadal timescales in response to rapidly changing environmental pressures (Egizi et al. 2015). This has implications for the common model fitting assumption that different mosquito collection datasets temporally far apart are comparable. The models do not account for any spatial and/or temporal heterogeneity in parameters beyond those fitted specifically to each dataset, nor do they offer a way of testing for such diversity, and this common assumption represents a limitation that also potentially undermines the modelling of other mosquito-disease systems.

Since the models fitted here contain a considerable number of parameters, as many as possible were based on existing laboratory or field data to fix them at biologically plausible values in order to contain the analyses and address the research aims as succinctly as possible. It should be noted, however, that all four models are also likely to be sensitive to variations in these parameters that arise due to on-going limitations in current entomological data quality and quantity; thus, although these parameters were assigned the most biologically realistic values (or environmental dependencies) based on the most up-to-date evidence and data on *An. gambiae* life history, further work is undoubtedly required to explore the implications of this additional parameter uncertainty on our findings. In this work, the decision was made to explore only the sensitivity of the models to those parameters that were not based upon experimental data, or for which the range of priors only were based on experimental data, in order to most clearly present the results and the implications for future modelling of *An. gambiae* (and other mosquito populations for which temperature- and age-dependence has been experimentally shown to exist, both in Chapter 2 of this thesis and in Clements & Paterson 1981; Styer et al. 2007; Styer et al. 2007; Harrington et al. 2008; Brady et al. 2013). However, as mentioned above, there are undoubtedly considerable gaps in the current entomological data (e.g. life-history parameter dependencies derived from a wide range of environmental variations, heterogeneity between different An. gambiae populations due to local adaptations, relatively small sample sizes in the limited number of controlled experiments that have been undertaken, and so on), and therefore the values at which non-fitted parameters were fixed may not be as optimally representative of the mosquito's biology as we have necessarily assumed. Further sensitivity or uncertainty analyses around these parameters is therefore likely to provide additional useful insight into the extent to which models can rely on current entomological laboratory or field data to inform their predictions and where future experimental and modelling studies are ideally required.

Some of the limitations described here may lead to the effect of senescence being underestimated in the model fits. Yet, despite the assumptions made, the models that include adult-only age-dependent mortality and larval and adult age-dependent mortality still fit the abundance data better than the other models, and it is therefore expected that the strength of the results concerning the importance of adult age-dependent mortality is very conservatively reported here. Reducing the uncertainty around some of the entomological parameters, and using more detailed datasets to inform model fitting and inference, may help to highlight further the true effect and importance of including age-dependent mortality, particularly in the adult stages, in models of vector abundance.

CHAPTER 6

DISCUSSION

In the context of the push to control and eliminate malaria (and other VBDs), there is a very important need to understand to what extent temperature and other environmental factors can realistically be expected to influence the population dynamics and geographical spread of disease vectors, and thereby the transmission of the diseases they transmit (Hunter 2011; Smith & Woodward 2013). This will allow better forecasting of which vector-control interventions are likely to have the greatest effect in areas of different climate profiles, and will enable predictions of intervention cost-effectiveness and success (Davis 2012; Parham & Hughes 2015). Knowledge of the impact of temperature on the mosquitoes responsible for transmitting malaria will also help inform and target further experimental investigation and more accurate and robust mathematical model predictions of malaria transmission scenarios, in particular under the alterations in environmental temperatures expected to occur under the influence of climate change and global warming (Githeko et al. 2000; Sutherst 2004; Mills et al. 2010; Waldock et al. 2013; Lotfy 2014; Roiz et al. 2014; Dhimal et al. 2015; Wu et al. 2016). In this thesis an experimental investigation is conducted into the extent to which temperature influences the life history of the mosquito *Anopheles gambiae* s.s., the principle vector of malaria in Africa, and, based on the experimental results, a theoretical framework is developed to examine to what extent it is important to include age- and temperature-dependent survival in models of *An. gambiae* population dynamics.

This chapter summarises the key results of this thesis, discusses the limitations of the thesis, and highlights the implications of the research as well as potential future objectives for further investigation in this field.

1. Summary of findings

In Chapter 2, I show, in keeping with previous studies, that the temperature of the environment has an effect on the survival of *Anopheles gambiae* s.s. mosquitoes, both during their larval stages and once they emerge as adults. However, I also show for the first time that the environmental temperature at which the immature stages develop affects the survival of adult mosquitoes, and that the magnitude of this effect may depend not only on the

environmental temperature of the larvae, but also on the temperature at which the adults subsequently live. In addition, by establishing that a Gompertz survivorship function yields the best fit to the experimental survival data and that a model of time-independent mortality fits the data significantly worse than other models that incorporate survival as time-dependent (and therefore age-dependent), I demonstrate that *An. gambiae* survival in the laboratory, both during the larval and the adult stages, is age-dependent, and that a constant hazard is not representative of the mosquito's biology.

In Chapter 3, I present further experimental results illustrating the effect of environmental temperature during the immature and adults stages on several other larval and adult life-history parameters of *An. gambiae* s.s. mosquitoes that determine mosquito abundance, and therefore have implications for disease transmission. My results indicate that environmental temperature affects mosquito size, developmental rates, probability of taking a blood meal, probability of ovipositing, and the number of eggs laid, but suggest that temperatures within the range I investigated (23°C-31°C) do not influence the time to oviposition once a blood meal has been ingested, nor the time to hatching once the eggs have been laid. Higher temperatures resulted in smaller larvae and adults, longer overall development times from eggs to adults, and a reduced probability of taking a blood meal and of oviposition, and there appears to exist an optimum window of temperatures within which the *An. gambiae* mosquito lays the most eggs. Some of the results detailed in chapter 3 confirm findings of previous studies, but the investigation into the effect of temperature during the immature stages on the life-history parameters of the adults is novel and the results show that the developmental temperature does indeed influence adult traits.

The model suite presented in Chapter 4 is based upon and supported by the experimental work presented in Chapter 2, showing that the main vector of malaria in humans on the African continent, *An. gambiae* s.s., does senesce, and Chapter 3, showing that several life-history parameters of *An. gambiae* depend on environmental temperature. The models are designed to determine whether including temperature-dependent and/or age-dependent survival provides any benefit when modelling the population dynamics of *Anopheles gambiae* s.s. This model suite involves a basic model in which mosquito survival is modelled as age-independent, and three more biologically realistic models in which survival is age-dependent in the adult stage only, in the larval stage only, or in the adult and larval stages. The model parameters, where possible, and the structure of the models with age-dependent survival, are informed by the data in Chapters 2 and 3: survival is modelled by a gamma-distributed

waiting time, which allows a computationally and mathematically straightforward model structure, where the stage whose survival is defined as age-dependent is modelled as a number of exponentially distributed sub-compartments. In addition, several parameters, including survival, in the models developed in this chapter can be set as temperature-dependent or temperature-independent.

The models developed in Chapter 4 were fitted to two longitudinal datasets of mosquito abundance, from Ghana and Tanzania, in Chapter 5, to determine whether modelling the mosquitoes' survival as age-dependent yields a better fit to data compared with the basic model in which survival is age-independent, and whether modelling mosquito life-history parameters, including survival, as temperature-dependent results in a better fit than assuming population dynamics to be temperature-independent. In most cases, assuming temperature-independent parameterisation appeared to yield a better fit than allowing the parameters to depend on daily temperature values. However, the two models with the best fit to data yielded a better fit to one dataset when assuming temperature-independent parameters, and a better fit to the other dataset when assuming temperature-dependent parameters. This chapter also shows that models comprising age-dependent survival in the adult stage fit the data significantly better than the model without adult age-dependent survival and better than the model with age-dependent survival only in the larval stage. A model including larval senescence as well as adult senescence did not fit the data better than a model including adult senescence only, which indicates that age-dependent survival in the larval stages may arguably be disregarded in the interest of model parsimony. These results were consistent across the two datasets, which indicates that the models are robust in their predictions.

2. Limitations

2.1. Limitations of the experimental work

While the experimental setup was carefully designed to answer the questions relevant to the modelling study in this thesis, certain sacrifices had to be made due to time and space constraints, and for the sake of experimental tractability.

The laboratory work that informed the results in Chapters 2 and 3 was limited to one species of mosquito, *An. gambiae* s.s.,, and explored the sensitivity to temperature of several life-history parameters within a wide temperature range (23-35°C), by following the development of the mosquitoes at four different temperatures with increments of 4°C. This difference in 4°C between treatments may be too low resolution to yield detailed information as to the

behaviour of mosquito populations between two data points, and any generalisation from the four temperatures examined here may not be representative of the mosquito's parameters at lower or higher temperatures, or at temperatures between the specific ones investigated. In addition, only the air temperature was controlled, meaning no information was gathered as to the water temperature of the larval environment. As the modelling results in Chapter 5 suggest that model output may be sensitive to the difference between air and water temperature, this information could have been useful and potentially relevant to the modelling study.

Only three functional forms were fitted to the survival data, in addition to the commonly used exponential function. While this was sufficient to demonstrate that *An. gambiae* s.s. mosquitoes do senesce in the laboratory, it may be that other functional forms may fit the data even better, with further implications as to age-dependent survival.

This particular experimental setup did not take into account other climatic factors such as humidity or possible desiccation, both of which have been shown to influence the survival of mosquito vectors (Warrell & Gilles 2002; Parham et al. 2012), nor did it incorporate diurnal/nocturnal temperature fluctuations. In addition, by rearing the larvae individually in 12-well plates, the effect of crowding, density-dependence, and cannibalism/predation on larval survival was ignored, which might have yielded a more realistic representation of the life history of mosquitoes in the field (Gilles et al. 2011; Muriu et al. 2013).

In the experimental setup described in Chapter 3, mosquitoes were blood fed seven days apart: this will have prevented any investigation into the effect of temperature on the time to blood feeding, which may be important for malaria transmission as, if higher temperatures shorten a mosquito's lifespan and interfere with their need to blood feed, this may impact the ability of the *Anopheles* female to transmit the parasite.

Finally, as all mosquitoes were censored at day 35, this may have obscured any additional effect that temperature may have on *Anopheles* life history parameters further down the line, as well as any long-term effects of the maternal environmental temperature on the life-history parameters of the first generation offspring.

These last points imply that while the experimental work in this thesis yields some brand new data as to how temperature affects *Anopheles* survival, life-history and reproductive parameters, and most importantly shows conclusively that mosquitoes do senesce, the experimental setup may not representative enough of *Anopheles gambiae* conditions in the field to allow these data to be used in informing predictions of mosquito's life cycle in the wild. For instance, while the results presented in Chapter 2 assert that age-dependent survival is a more realistic assumption for mosquito mortality than time-independent survival, there

are conflicting expectations as to whether vectors in the wild may live long enough for the effects of age-related mortality to be noticeable (Ryan et al. 2015)

2.2. Limitations of the model development and fitting

By its nature, mathematical modelling involves a simplification of the phenomenon modelled to include only those characteristics that are important and useful in answering the specific question the model seeks to answer. This typically leads to a number of biological and epidemiological assumptions and compensations in order to make the model more tractable.

Some difficulties arise from the limited availability of good quality datasets to enable robust model fitting. While longitudinal An. gambiae abundance data exist for several locations across sub-Saharan Africa at different climates, altitudes, and within different environments, these vary widely in terms of duration, ease of availability, provision of sufficient detail to enable robust model fitting, and overall data quality. Very few datasets describe monthly-resolution mosquito abundance for a period longer than 2 years (which would facilitate consistent vector behaviour and climate-driven population response to emerge as distinct temporal patterns, and thus would increase the likelihood of a good fit to the data (Churcher et al. 2015)). The quality of available datasets, and the level of detail with which abundance data are recorded or made available, strongly influence the ability to fit models to these abundance datasets, and, hence, the accuracy of the models' predictions. This unavoidably results in model fitting requiring a priori assumptions, for example about the mean and standard deviation around the total monthly number of catches. In addition, as the models were only fitted to two datasets, this may not be representative of the models' performance at large; in particular, the results of including temperature-dependence in the models were inconclusive, and a large sample of datasets to fit to might have yielded a better insight into the extent to which temperature-dependence influences model fitting. It may also be that this manner of including temperature-dependence in the models was not optimal, and that another model structure may lend itself better to this task.

While the experimental results detailed in Chapter 2 suggest that the Gompertz functional form best describes the survival data, age-dependent survival was depicted using the gamma functional form in the models here. While the fit of the gamma function to the survival data appears to justify this assumption, incorporating Gompertz-described mortality may lead to a different model output, with different implications for the effect of incorporating age-dependent survival in vector models. One possible element of uncertainty introduced into the modelling component of this thesis comes from the use of the gamma function to describe age-dependent mortality, rather than the Gompertz function, which was shown in Chapter 2 to

fit the data best. While the gamma function did not fit significantly worse than the Gompertz function overall, and in some cases fitted better, the fact that the Gompertz did yield the best fit overall suggests this might have been a better function to use to depict age-dependent mortality in the models. The gamma function was chosen here over the Gompertz as it allowed the use of ODE models – a simple modelling setup based on the Ross-Macdonald framework – and it allowed the incorporation of age-dependent survival as part of the model structure, while modelling Gompertz-described senescence would be much more computationally or mathematically intense. In addition, as the rainfall-dependent density function included in the models here was based upon that by White et al. (2011), modelling the population dynamics in the manner described in Chapter 4 meant that the baseline model (with no temperature- or age-dependent survival) is very close to that developed by White et al., which was shown to fit well to different datasets gathered as part of the Garki project (White et al. 2011).

Another element of uncertainty introduced by modelling age-dependent survival as a gamma function involved fixing the number of compartments: in order to incorporate the gamma function into the model structure, the parameter describing the number of compartments (the shape parameter, α) had to take on one fixed value for each model. However, I show in Chapters 2 and 4 that senescence is age-dependent, so that fixing the parameters of the gamma function may (and in some cases, did) lead to a worse fit of the gamma function to the data: this suggests that the framework presented here for modelling age-dependent survival, while an improvement on models describing survival as age-independent, may not be the best method.

The importance of detailed and comprehensive data in informing models has been emphasised previously (Reiner et al. 2013), and this thesis confirms the importance of basing mathematical models on solid empirical data, by showing that models including biologically realistic age-dependent survival fit abundance data considerably better than models representing mortality as age-independent. However, models incorporating survival as age-independent follow the precedent set by Macdonald (Macdonald 1952), who assumed that mosquitoes in the wild were more likely to die of adverse environmental conditions, disease, or predation than of old age. While it appears that more and more lab data and statistical models are highlighting the existence of age-dependent survival (Dawes et al. 2009; Styer, Carey, et al. 2007), until senescence is confirmed as a valid model of survival in the wild the use of lab data to inform models and predictions may be erroneous.

The fitting yielded different quality fits to the two datasets: in both cases the models that included adult senescence fit the data considerably better than the models that did not, but the fit to one of the datasets was noticeably worse than to the other. In addition, it is surprising that the models that included temperature-dependence did not yield significantly better fits than the models that did not, given the significant experimental evidence that mosquito life-history parameters are dependent on environmental temperature (Impoinvil et al. 2007; Huang et al. 2006; Kirby & Lindsay 2009; Depinay et al. 2004; Kirby & Lindsay 2004; Afrane et al. 2007a; Midega et al. 2007; Olayemi & Ande 2008; Lunde et al. 2013). This may signify that the models are not flexible enough to capture the intricacies of different datasets accurately, or that another fitting algorithm might be better suited to improving the performance of these models. Further research is needed, perhaps into fitting these models with a different algorithm (such as the more traditional basin-hopping algorithm mentioned in Chapter 5), or into different model structures (as the representation of age-dependent survival through a fixed number of compartments may remove some of the flexibility to describe temperature dependence).

One final source of uncertainty involves the difficulty in obtaining reliable, consistent longitudinal climate data. Temperature data proved easier to find than precipitation data, but even this has not been optimised for the purpose of localised modelling: datasets of vector abundance rarely provide the climate data information to match their surveillance data, and so modellers rely heavily upon regional climate models to provide the data necessary to run their abundance or transmission models. This however presents its own problems, as when modelling vector population dynamics or disease transmission, one may typically require spatial resolution at the village or household level, while regional climate models tend to provide a much lower spatial resolution, usually 25-50km. This may lead to considerable uncertainty in the model output, and may result in a poorer fit to data than might have been expected.

3. Implications of the findings

Basing models on the results of experiments designed specifically to inform model structure and parameterisation, as was the case for the models developed in Chapter 4 and the experiments in Chapters 2 and 3, is a first step towards the development of more detailed and robust frameworks that will allow a better understanding of the effects of climate on the distribution and density of mosquito vectors of disease. In this case, the experimental work first suggested that senescence does occur in *An. gambiae* mosquitoes, and this result was

validated by the modelling study, which confirmed that population dynamics models that include age-dependent mortality fit surveillance data better than models that ignore it. This suggests that models of vector abundance that assume a time-independent hazard (characterised by age-independent mortality) may not be representative of the mosquito's biology, and may also fail to capture some of the intricacies of the behaviour of the mosquito population. The importance of combining experimental studies with theoretical work is further emphasized here as the results indicate almost no difference in model fitting between age-dependent survival in the adult stages only, and age-dependent survival in both the larval and adult stages. This highlights another important conclusion of this thesis: despite experimental evidence for both larval and adult age-dependent mortality, including age-dependent mortality in the adult stages only represents both the most parsimonious option for modelling and the best fit to data for the models developed here. This also emphasises the need for comprehensive experimental studies

In addition to furthering the understanding of vector population dynamics, more biologically or entomologically accurate vector models based on experimental data may help inform more reliable disease transmission models. For instance, since the inclusion of adult senescence in models of mosquito abundance appears to enable more accurate and reliable predictions of the population dynamics of a key malaria vector in sub-Saharan Africa, this may have important implications for not just for the geographical distribution of the vector, but also for modelling disease spread – in this case, the spread of malaria, but possibly applicable to other vectors of VBDs as well.

The modelling frameworks developed here are not limited to *An. gambiae*, or indeed to malaria. While the particular models created here are only relevant for *Anopheles gambiae* s.s. (due to the parameterisation based on entomological data of this species alone), dozens of other *Anopheles* species also transmit malaria (Sinka et al. 2012). There is currently no detailed experimental evidence to assess whether the life-history parameters of one Anopheles species may be applicable to others, and this highlights the need for extremely precise experimental data on the many vectors of VBDs. As *An. gambiae* is the most common vector of malaria in Africa (Sinka et al. 2012), it is likely that the availability and quality of data on this species is higher than for other, less common species (Okorie et al. 2011). However, depending on the availability of detailed life-history parameters for other mosquito or insect species, the framework developed here is readily applicable to other vector species to assess the importance and role of temperature- and age-dependent mortality on vector abundance and, ultimately, disease transmission and implications for control.

An important point leading on from this thesis is the expected role that climate change may play in the distribution of *Anopheles gambiae*, and therefore of the spread of malaria. The Fifth Assessment Report by the Intergovernmental Panel on Climate Change (IPCC 2013) for the near-term climate (defined as the period spanning 2016 to 2035) suggests that the average global surface air temperature will increase by approximately 0.3°C–0.7°C, and that there will be an increase in the duration, intensity, and spatial reach of heat-waves. Air temperatures currently vary broadly across Africa, with night-time air temperatures ranging from 6°C to 29.5°C, and daytime air temperatures from 17°C to 41.3°C (Garske et al. 2013). This implies that the sensitivity of *An. gambiae* s.s. to changes in environmental temperature is likely to be extremely region-specific and that any predicted short-term changes in temperature may not strongly influence *An. gambiae s.s.* distribution in areas where this mosquito is already established and present. The results presented in Chapters 2 and 3 appear to support this, by showing that small changes in temperature are less likely to affect survival than larger fluctuations.

The impact of climate change will vary greatly according to geographical location (Ermert et al. 2012; Linard & Tatem 2012; Linard et al. 2012; Tatem et al. 2012; Tay et al. 2012) is expected to impact significantly the distribution of malaria in areas with climatic characteristics close to the physiological tolerance of the mosquito and parasite, such as the southern limit of malaria in South America, by affecting the distribution of *Anopheles darlingi* (Carcavallo & Curto de Casas 1996). This, however, is disputed by predictions suggesting that malaria is unlikely to change even under extreme climate scenarios, due to the complexity of the parasite lifecycle, and the many stages affected by environmental variables (Rogers & Randolph 2000; Shiff 2002), as well as the possible geographical redistribution of *Anopheles* species (Tonnang et al. 2010).

As highlighted in Chapter 1, the accuracy of malaria transmission predictions under different climate scenarios depends heavily on the reliability of current data on climate trends, vector entomology, parasite biology, and healthcare coverage. There is currently substantial uncertainty around the effects that climate change will have on malaria transmission (Piontek et al. 2014; Jones & Morse 2010), partly because of a lack of precise data on the sensitivity of the vector and the parasite to climate-related factors (Roiz et al. 2014), and partly due to the unknown course climate change will take and the unknown effects of global warming. It is predicted that climate change will bring with it an increase in extreme weather events, including flooding, drought, and particularly tropical storms (Morse et al. 2009; Parham et al. 2011; IPCC 2013). These events will likely lead to changes in land use, which, along with

deforestation and vegetation clearance, are expected to influence the spread of malaria in uncertain ways that will depend on species' sensitivity to environmental factors (Lindsay & Birley 1996), as well as human-mediated factors.

4. Relevance for future research

Future work based upon the experimental data provided in this thesis may want to explore the importance of including the effect of larval temperature on adult life history parameters: Chapters 2 and 3 show that the temperature to which the larvae are exposed during their development influences adult survival as well as other reproductive parameters. This may provide a basis for further modelling work as well, in that it is shown here that the manner in which adult survival is modelled has a considerable impact upon model predictions: while Chapter 5 indicates that larval survival appears to be less important a factor in predicting *Anopheles gambiae* s.s. abundance than the manner in which adult survival is depicted, it may be that the larval environment, by influencing adult survival, has a significant impact on population dynamics. In addition, other parameters such as larval development, and the probability that an adult female mosquito blood feeds, are shown here to depend on temperature, but are not incorporated into the models.

In addition, it may be useful to fit the models developed here, or the framework upon which they are built, to more datasets of abundance, with climate data obtained from the same source (two different sources of climate data were used to match the two abundance datasets used in Chapter 5), to determine whether the important effect of including age-dependent survival is still consistently significant, and therefore whether the model framework does indeed highlight a need to include age-dependent survival in models of mosquito population dynamics.

Due to the model structure limitations, alternative model structures may be more appropriate for modelling temperature-dependent development and mortality, into which the concept of temperature- and age-dependent may be more readily and naturally incorporated without having to limit the model structure. One such structure is the degree-day (DD) formulation, which is particularly applicable for modelling insects and pests as it models physiological age, as opposed to chronological age (Jones & Brunner 2015), and expresses the development of poikilotherms as a function of the difference (in degrees Celsius) between the environment and the minimum temperature for development (MTD) of the insect (Young & Young 2002; Ahumada et al. 2004; Jones & Brunner 2015). For each mosquito stage, one degree-day (DD)

is defined as a period of 24 hours (1 day) during which the environmental temperature is 1 degree Celsuis (1°C) above the minimum temperature for development (MTD) of that mosquito stage (Young & Young 2002). This framework is extended so that if the environmental temperature is 2°C above the MTD for 24 hours, 2 DD are accrued, if it is 3°C above MTD for 24 hours, 3 DD are accrued, etc. While such models are not yet widespread in the field of vector-borne diseases, they are commonly used in the field of pest management and insecticide treatment (Baker et al. 2000; Young & Young 2002), as well as agriculture (Schlenker & Lobell 2010) and glaciology (Braithwaite & Raper 2007), but has the scope to be hugely useful in the field of VBD modelling, where the insect vectors depend intrinsically on environmental temperature.

This highlights another potential for future research: as temperature- and age-dependent survival may be modelled with other model structures than the one presented here and with other parameterisations (such as the Gompertz function, shown to fit the data best), it may be useful to perform a model comparison to rate the performance of different model structures and parameterisations, as well as to quantify and compare the uncertainty arising from the assumptions made by each model.

Finally, *Anopheles gambiae* s.s. is only one of the seven major vectors of human malaria in Africa (Sinka et al. 2012) and available data on the sensitivity of the other species to climatic variables such as temperature, as well as population-related factors, are even more scarce than data on *Anopheles gambiae* s.s.. Climatic and environmental changes are expected to influence the reproductive and life-history parameters (Lyons et al. 2013) of different mosquito species in different ways (Sinka et al. 2012). As different species of mosquito and insect vectors are likely to respond differently to changes in regional and temporal temperature patterns, more comprehensive data specific to each malaria vector species are required to define the dependency of mosquitoes' population dynamics on climate-related variables, which, combined with regional climate data predictions and comprehensive and detailed data on disease transmission, will allow more sound and dependable forecasting of disease vector population dynamics, and thereby of transmission patterns.

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APPENDICES

Appendix A

Christiansen-Jucht *et al. Parasites & Vectors* 2014, **7**:489
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Temperature during larval development and adult maintenance influences the survival of *Anopheles gambiae* s.s.

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Abstract

Background: Malaria transmission depends on vector life-history parameters and population dynamics, and particularly on the survival of adult *Anopheles* mosquitoes. These dynamics are sensitive to climatic and environmental factors, and temperature is a particularly important driver. Data currently exist on the influence of constant and fluctuating adult environmental temperature on adult *Anopheles gambiae* s.s. survival and on the effect of larval environmental temperature on larval survival, but none on how larval temperature affects adult life-history parameters.

Methods: Mosquito larvae and pupae were reared individually at different temperatures (23 ± 1°C, 27 ± 1°C, 31 ± 1°C, and 35 ± 1°C), 75 ± 5% relative humidity. Upon emergence into imagoes, individual adult females were either left at their larval temperature or placed at a different temperature within the range above. Survival was monitored every 24 hours and data were analysed using non-parametric and parametric methods. The Gompertz distribution fitted the survivorship data better than the gamma, Weibull, and exponential distributions overall and was adopted to describe mosquito mortality rates.

Results: Increasing environmental temperature during the larval stages decreased larval survival ($p < 0.001$). Increases of 4°C (from 23°C to 27°C, 27°C to 31°C, and 31°C to 35°C), 8°C (27°C to 35°C) and 12°C (23°C to 35°C) statistically significantly increased larval mortality ($p < 0.001$). Higher environmental temperature during the adult stages significantly lowered adult survival overall ($p < 0.001$), with increases of 4°C and 8°C significantly influencing survival ($p < 0.001$). Increasing the larval environment temperature also significantly increased adult mortality overall ($p < 0.001$): a 4°C increase (23°C to 27°C) did not significantly affect adult survival ($p > 0.05$), but an 8°C increase did ($p < 0.05$). The effect of a 4°C increase in larval temperature from 27°C to 31°C depended on the adult environmental temperature. The data also suggest that differences between the temperatures of the larval and adult environments affects adult mosquito survival.

Conclusions: Environmental temperature affects *Anopheles* survival directly during the juvenile and adult stages, and indirectly, since temperature during larval development significantly influences adult survival. These results will help to parameterise more reliable mathematical models investigating the potential impact of temperature and global warming on malaria transmission.

Keywords: *Anopheles gambiae sensu stricto*, Environmental temperature, Larval survival, Mosquito survival, Climate change

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Background

Although historical data and theoretical models suggest that the distribution of malaria is much more sensitive to the scale-up of control measures than to climate change, it appears evident that climate change will affect the distribution and transmission of mosquito-borne diseases such as malaria [1] and thereby influence the extent to which the disease can be controlled. However, we currently have a limited understanding of how climatic factors affect the entomological parameters determining transmission. The most obvious question is how increasing temperatures associated with climate change will affect mosquito longevity and the duration of the parasite's development within the mosquito, two of the most influential parameters underlying the transmission of mosquito-borne diseases.

However, temperature also shapes mosquito life-history traits that are associated with vector-competence and determines mosquito population density: a warmer environment leads to faster development and smaller adults. Mosquito size can influence epidemiologically relevant traits such as longevity, length of the gonotrophic cycle, immunocompetence, size of the bloodmeal (and probability of infection), biting rate, and intensity of infection. These traits in turn can affect mosquito survival [2] and parasite development [3]. The effect of temperature on mosquito life-history might also affect transmission by influencing fecundity, which is limited by size. Moreover, mosquito population density and fecundity feedback through larval density to influence the development of mosquitoes by density-dependent competition and mortality [4]. Although sketches of these interactions are known, their integration is lacking but essential to allow us to predict how temperature may influence malaria transmission. In this paper, we focus on the effect of temperature on mosquito survival.

Human malaria is transmitted via the bites of female *Anopheles* mosquitoes. Mosquitoes need to bite at least twice to acquire and transmit the infection, and the *Plasmodium* parasites undertake a complex sporogonic cycle within the vector, such that depending on environmental temperature, the duration of the extrinsic incubation period can be similar to the average life expectancy of the mosquito [5]. This makes malaria transmission particularly vulnerable to the daily survival probability of the vector, since it is necessary for mosquitoes to survive until completion of sporogony and beyond this in order to transmit salivary gland sporozoites to susceptible hosts.

Anopheles mosquitoes are sensitive to mean environmental temperature as well as its temporal fluctuations. Understanding how temperature influences vector ecology is therefore extremely important in predicting mosquito distribution as well as vector fitness and capacity to transmit malaria [6]. This understanding of the factors affecting vector populations will also improve projections of future malaria transmission, as environmental shifts due to climate change are likely to affect the global spread of malaria [7], and in particular, climatic factors that influence vector survival are likely to influence malaria transmission [8]. However, the magnitude of the vector population dynamics dependence on climatic factors remains uncertain [7-9].

Mosquito survival has been shown to depend on temperature, rainfall, and humidity [10], as well as other factors such as mosquito density [11,12], genetic diversity [13], and the ability to find a blood meal. Data have been reported on the influence of adult temperature on adult survival [14-18]. Fewer data exist on the influence of juvenile environmental temperature on juvenile survival [19-23], but none exist, to our knowledge, on the influence of environmental temperature during the juvenile stages on adult mortality, although temperature throughout the mosquito's development may have repercussions on its survival [24].

This report presents the results of an experimental investigation into the influence of environmental temperatures during the *Anopheles* mosquito's juvenile and adult stages on survival. It has been suggested that the maternal environment has an influence on the population dynamics of *Anopheles* mosquitoes through its impact on offspring development, survival, and susceptibility [25].

Methods

Larval maintenance and temperature regimes

Anopheles gambiae sensu stricto (*s.s.*) mosquitoes, originating from the Kisumu colony from Western Kenya, were maintained at Imperial College London's Silwood Park campus. Two days after hatching, larvae were individually placed in 12-well plates with 3 mL of deionised water, at one of the following environmental (air) temperatures: 23 ± 1°C, 27 ± 1°C, 31 ± 1°C, and 35 ± 1°C. For every temperature, 640 larvae were reared at a food regime of TetraMin® baby fish food until development into imagoes. On day 2 after hatching, larvae were given 0.02 μg of baby fish food per 100 mL of de-ionised water; on days 3, 4, 5, and 6, they were given 0.06, 0.08, 0.16, and 0.32 μg per mL respectively; and on days 7 until pupation, 0.6 μg per mL.

Mosquitoes were reared in a 12:12 light/dark cycle, at 75% (±5%) relative humidity (RH). Larvae were checked every 24 hours to count the number of dead and live, and to construct life-tables.

As each larva was reared individually, each mosquito was considered an individual data point. Our data is therefore representative only of the mosquito colony used in this experiment, and confirmation of our results

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is ideally required in other mosquito species, experimental, and field settings.

Adult maintenance and temperature regimes

Upon emergence into adults the mosquitoes were divided into three groups and each group was placed at either 23°C, 27°C, or 31°C (see Figure 1). This allowed the distinction between the effects of larval and adult environmental temperatures on adult survival. All larvae reared at 35°C died as immature stages and it was therefore decided not to maintain any adults at 35°C.

Adults were given four days to mate, before the females were placed in individual plastic cups and given a 10% sugar solution, while the males were discarded. Females were blood fed on CC-J's arm on three occasions: 5, 12, and 19 days after emergence as adults. The time between blood meals was set as 7 days to allow all females to lay eggs (and the eggs to hatch). The sugar solution was removed 24 hours before each blood meal to ensure that females were eager to feed. Females that did not feed were discarded.

The bottom of each cup was filled with deionised water 24 hours after each blood meal to allow the females to lay eggs, and the mosquitoes were transferred to new, dry cups 48 hours after laying eggs. Adult survival was measured every 24 hours. All dead and live females were counted and the results recorded for the construction of life-tables. Censoring occurred 33 days after hatching, with all mosquitoes monitored until that day. On day 33, all mosquitoes still alive were frozen and their wing length measured.

In this report, only the survival data are presented. Data on larval developmental rates, adult female fecundity (number of eggs laid), fertility (number of eggs hatched), and mosquito size (measured by wing length) will be presented elsewhere.

Statistical methods

Survival analysis

Non-parametric methods Survival analyses were performed on each juvenile/adult temperature combination using Kaplan-Meier analysis [26], as this is a standard non-parametric method of representing survival data, and enables a useful comparison with data sets from similar experiments elsewhere to be made. The difference between results from different temperature regimens was compared using the log-rank and Mantel-Cox tests, both standard methods to test the null hypothesis that survival functions do not differ across groups. The log-rank test was used to compare the overall survival trend for the range of temperatures explored [27], and the Mantel-Cox test was used for two-sample comparisons of survivorship at one temperature against the survivorship at the baseline temperature (23°C) [27,28]. The results are given as a test statistic, which was compared with a Chi-squared distribution with one degree of freedom to yield a p-value. Mosquitoes killed on day 33 were classified as censored observations. The median survival time (with 95% confidence intervals) was calculated for each group to compare survival times, by determining the time beyond which 50% of the individuals in the population were expected to survive [27].

Parametric methods In order to test the widely-applied assumption that adult *Anopheles* survival follows a model of constant mortality, the exponential, gamma, Gompertz, and Weibull survival functions [27] were fitted to larval and adult survival data at each temperature regimen by maximum likelihood estimation (MLE). The exponential model implies a constant mortality rate, whilst the remainder allow for age-

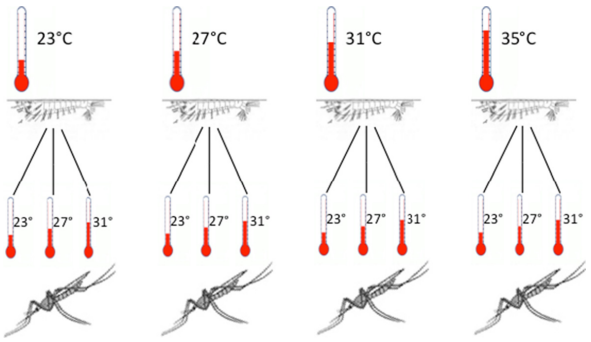


Figure 1 Experimental design. Larvae (640) reared at each temperature (23°C, 27°C, 31°C, 35°C) were allowed to develop into imagoes, and the adult females were kept at the same temperature at which they were reared as juveniles, or placed at one of the other two temperatures. None of the larvae reared at 35°C survived to adulthood, so no adults were maintained at that temperature.

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(or time-) dependent mortality. In the log-likelihood function (log-*L*),

$$\log L = \sum_t N_t S(t) + (N_0 - N_t)(1 - S(t)) \quad (1)$$

N_0 is the number of mosquitoes (larvae or adults) alive at the beginning of the experiment, N_t is the number alive at the beginning of day t, and $S(t)$ is the probability of surviving to day t according to the fitted survivorship function. Goodness-of-fit was compared using the Akaike Information Criterion (AIC), or by AICc (corrected Akaike Information Criterion) when the sample size was smaller than 80, to avoid over-fitting [29,30]. According to [29], a difference of ≤2 in AIC values indicates the two fits are not significantly different and only models with a difference of >4 in AIC values are statistically distinguishable. The Gompertz survival function,

$$S(t) = \exp\left\{\frac{\lambda}{\theta}[1 - \exp(\theta t)]\right\} \quad (2)$$

was found to fit the survival data better than the exponential, gamma and Weibull survival functions in 10 out of 16 temperature scenarios, and was not significantly worse than the best fit in 2 further cases (Additional file 1: Table S1, Additional file 2: Table S2, Additional file 3: Table S3 and Additional file 4: Figure S1). This has implications for modelling *Anopheles* population dynamics and malaria transmission, as it suggests an age-dependent mortality model for the adult stages is more appropriate than assuming constant mortality. The best-fit Gompertz parameter values obtained by MLE at each environmental temperature were used in the corresponding hazard function

$$h(t) = \lambda \exp(\theta t) \exp\left\{\frac{\lambda}{\theta}[1 - \exp(\theta t)]\right\} \quad (3)$$

to describe larval and adult mortality at all temperatures regimes tested.

Uncertainty around the two Gompertz parameters at each larval and adult temperature was calculated using the profile likelihood method [31].

The non-parametric analyses were carried out using R, Version 2.10.1 (R Foundation for Statistical Computing, 2009), while Microsoft Excel (Microsoft Office 2008) was used for the parametric analyses and uncertainty calculations.

Results

The effect of larval environmental temperature on larval survival

Of the four larval groups, it was only possible to estimate the median survival time (8 days) for those reared at 35°C (Additional file 5: Table S4); for all lower temperatures, the survivorship curves did not cross the value of 0.5 (Figure 2A). According to the Kaplan-Meier plots, larval mortality increased notably with increasing environmental temperature (Figure 2A). The overall trend showed a statistically significant increase in mortality with increasing temperature ($p < 0.001$) (Additional file 6: Table S5). The decrease in larval survival was statistically significant for a 4°C increase in temperature (from 23°C to 27°C ($p < 0.001$)), as well as for an 8°C increase from 27°C to

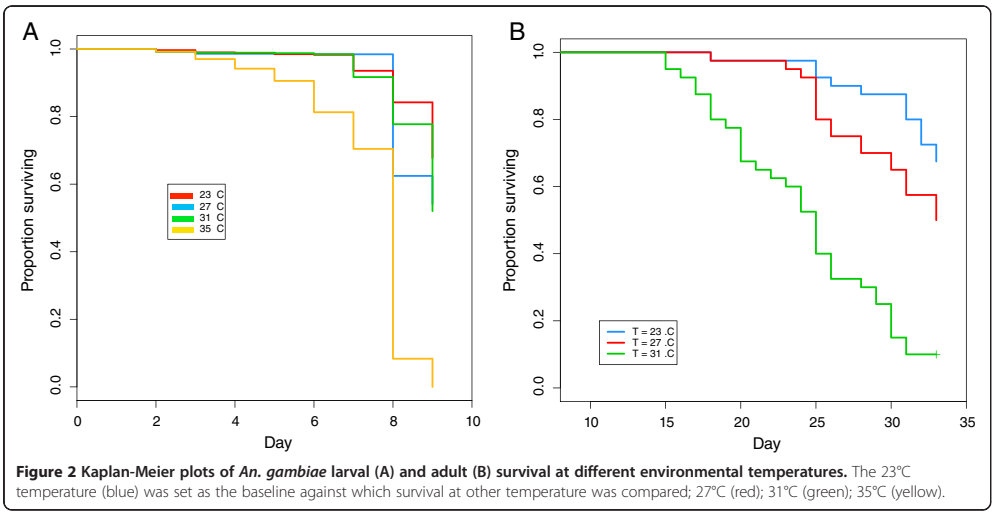


Figure 2 Kaplan-Meier plots of *An. gambiae* larval (A) and adult (B) survival at different environmental temperatures. The 23°C temperature (blue) was set as the baseline against which survival at other temperature was compared; 27°C (red); 31°C (green); 35°C (yellow).

35°C ($p < 0.001$), and a 12°C increase (from 23°C to 35°C, $p < 0.001$). The data resulting from an 8°C increase in environmental temperature from 23°C to 31°C did not allow us to perform a meaningful statistical test.

Decreases in larval survival were also statistically significant when the 4°C increases referred to temperatures other than the baseline; increases from 27°C to 31°C, and from 31°C to 35°C, both resulted in statistically significant increases in larval mortality with $p < 0.001$. All larvae reared at 35°C died before emergence into adults.

The effect of adult environmental temperature on adult survival

Additional file 7: Table S6 and Additional file 8: Figure S2B indicate that, although the survivorship curve did not cross 0.5 for adult female mosquitoes maintained at 23°C, and it was therefore not possible to calculate the median survival time at this temperature, median survival decreased from 31 days (at 27°C) to 25 days (at 31°C). Overall, higher environmental temperatures were statistically and positively associated with an increase in adult mortality ($p < 0.001$) (Additional file 9: Table S7 and Figure 2B). The mortality experienced by adult mosquitoes was strongly and significantly more elevated with every increase in temperature relative to the baseline of 23°C, i.e. p-values were all highly significant ($p < 0.001$) for comparisons of 27°C vs. 23°C and 31°C vs. 27°C (each a 4°C increase), as well as for 31°C vs. 23°C (8°C increase, Additional file 9: Table S7).

The effect of larval environmental temperature on adult survival

Table 1 summarises the median survival times for each group of adult temperatures and the temperatures at which these adults had been reared as larvae. In general, there is a trend for decreasing median survival times of adult females with increasing adult environmental temperature. Within each group, for similar larval and adult environmental temperatures, median survival times tend to be higher than when larvae and adults are maintained at more divergent temperatures; for instance, when both larvae and adults are exposed to 31°C, median survival time is 26 days, but only 22 days when the larvae had been reared at 23°C.

Environmental temperature during the larval stages was found to have a marked effect on the survival of adult mosquitoes. When the mosquitoes reared at different temperatures were placed at 23°C as adults, those who had been reared at 27°C as larvae did not experience a significantly higher mortality than those reared at a larval temperature of 23°C ($p = 0.92$). However, those mosquitoes who were reared at 31°C had a higher mortality than larvae reared at 27°C ($p < 0.05$), and those mosquitoes that had been exposed to an 8°C decrease (from 31°C to 23°C) suffered a statistically significant increase in mortality compared to 23°C ($p < 0.05$) (Figure 3A and Table 2).

When larvae reared at different temperatures (23, 27, 31°C) were moved to 27°C as adults, those who had also been reared at 27°C did not experience a significant decrease in adult survival compared with those reared at 23°C ($p = 0.927$), while those exposed to a 4°C decrease between the larval and the adult stages (from 31°C as juveniles to 27°C as adults) experienced a statistically significant reduction in adult survival ($p < 0.001$) (Figure 3B and Table 2).

Finally, when adults were kept at 31°C, mosquitoes experiencing a 4°C increase in temperature (from 27°C to 31°C) were not observed to have a significantly affected survival ($p = 0.182$), but an 8°C increase (from 23°C to 31°C) significantly increased adult mortality ($p < 0.01$). The overall influence of larval temperature on adult survival was significant when adults were maintained at 23°C ($p < 0.05$), 27°C ($p < 0.001$), and 31°C ($p < 0.05$) (Figure 3C), while the overall effect of larval environmental temperature on adult survival, at

Table 1 Median survival times of adult *An. gambiae* s.s. according to the temperature of the adult environment, and the temperature at which the larvae that developed into such adults had been reared

Adult temperature (°C)	Larval temperature (°C)	Total number of adults exposed at the start	Median adult survival (days) (95% C.I.)
23 ± 1	23 ± 1	39	ND*
	27 ± 1	40	ND*
	31 ± 1	24	32.0 (30, ND)
27 ± 1	23 ± 1	40	33.0 (31, ND)
	27 ± 1	40	33.0 (31, ND)
	31 ± 1	40	28.5 (25, 30)
31 ± 1	23 ± 1	26	22.0 (19, 25)
	27 ± 1	40	25.0 (22, 28)
	31 ± 1	23	26.0 (25, 30)

*ND: Not determined. Median survival defines the time point at which the survivorship curve crosses 0.5, or at which 50% of the sample is expected to survive. In this case, the survival function did not cross 0.5, and the median survival cannot be calculated.

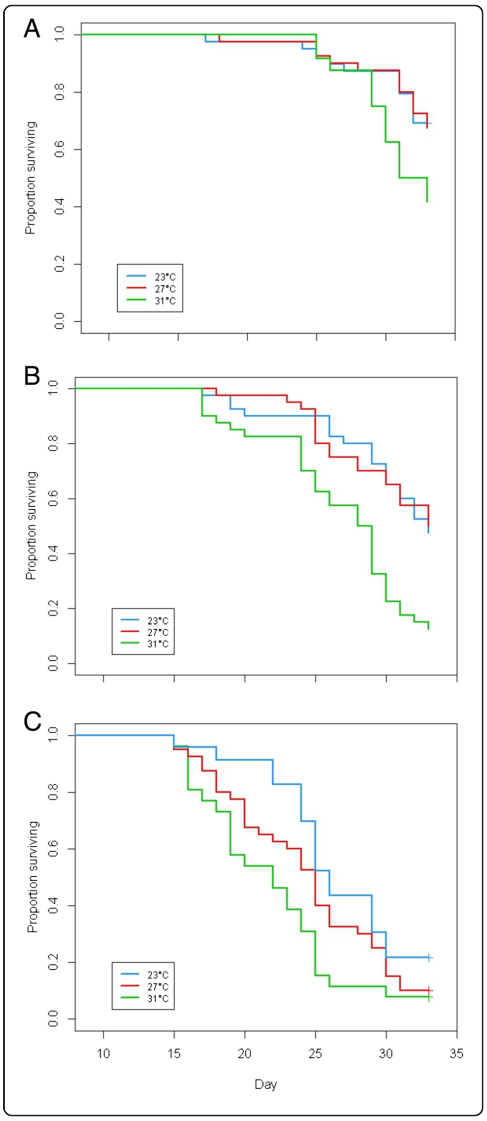


Figure 3 Kaplan-Meier plots of *An. gambiae* adult survival at different environmental temperatures having been reared as larvae at different temperatures. (3A). Adult survival curves at adult environmental temperature of 23°C. Larval temperature 23°C (blue) was set as the baseline against which survival at other larval temperatures was compared; 27°C (red); 31°C (green). **(3B)**. Adult survival curves at adult environmental temperature 27°C. Larval temperature 23°C (blue) was set as the baseline against which survival at other larval temperatures was compared; 27°C (red); 31°C (green). **(3C)**. Adult survival curves at adult environmental temperature 31°C. Larval temperature 23°C (blue) was set as the baseline against which survival at other larval temperatures was compared; 27°C (red); 31°C (green).

all adult temperatures, was highly significant ($p < 0.001$) (Table 2).

Parametric curve fitting to survival and mortality data

Additional file 8: Figure S2 and Additional file 10: Figure S3 show the best-fit Gompertz survival curves for each combination of larval and adult temperatures. The values for the two parameters of the Gompertz survival function, λ and θ, and their 95% confidence intervals (CI) at each temperature are shown in Additional file 11: Figure S4. These values were used to develop Gompertz hazard functions, which were plotted against the mortality data for each temperature regimen (Additional file 12: Figure S5 and Additional file 13: Figure S6). Additional file 13: Figures S6a and S6b show that both parameters of the Gompertz survival functions vary widely as a function of larval temperature, whereas θ only changes significantly with respect to adult environmental temperature. Additional file 13: Figure S6c shows that for the Gompertz curves describing adult survival at adult temperatures of 23°C and 27°C, both λ and θ remain relatively invariant with respect to the temperature at which the larvae were reared, but change with larval environment temperature when adults were maintained at 31°C.

Discussion

This study demonstrates that environmental temperature affects the survival of *Anopheles gambiae* s.s., both during their immature stage development and during their lifetime as adults. Results of the larval survival experiments indicated a statistically significant decrease in larval survival with every 4°C increase in environmental tem perature, in agreement with previous studies [19,20,32]. Similarly, there was a statistically significant decrease in adult survival with each 4°C increase in environmental temperature, as has also been reported elsewhere [23,33,34].

However, this is the first study to investigate the effect of larval temperature on adult *Anopheles gambiae* survival. Our results indicate that a small difference (4°C) between the larval and adult temperatures may have a significant impact on adult survival, and this may depend on the temperature at which this difference occurs. This suggests that the temperature of the larval environment may have a much more important impact on the adult stages than was previously thought. Due to the complexities of the experimental setup and logistical constraints

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Table 2 Two-group comparisons and overall trend of the effect of increasing larval environmental temperature on the survival of adult *An. gambiae* s.s. mosquitoes, at different adult environmental temperatures

		Larval temperature (°C)			Overall effect of larval temperature on adult survival	
Adult temperature (°C)	**Test statistic**	**27 ± 1 (with respect to 23°C)**	**31 ± 1 (with respect to 23°C)**	**31 ± 1 (with respect to 27°C)**	**Test statistic**	
23 ± 1	Mantel-Cox test	0.01	4.88	4.63	Log-rank test	6.51
	p-value	0.920	0.027	0.031	p-value	0.039
27 ± 1	Mantel-Cox test	0.01	16.29	19.43	Log-rank test	23.51
	p-value	0.927	<0.001	<0.001	p-value	<0.001
31 ± 1	Mantel-Cox test	2.74	7.41	1.78	Log-rank test	7.61
	p-value	0.098	0.006	0.182	p-value	0.022
All adult temperatures					Log-rank test	108.30
					p-value	<0.001

as to the number of mosquitoes that could be reared and observed, only four temperature treatments and only 4°C increases were investigated here. It is, therefore, difficult to extrapolate our conclusions to more nuanced increases in temperature.

In general, the Gompertz survivorship function fitted the survival data reasonably well, confirming the results of Clements and Paterson [35] and indicating the operation of age-dependent mortality (senescence) in both immature and adult stages, at least under laboratory conditions. Dawes *et al.* also reported age-dependent mortality in laboratory adult populations of *An. stephensi* [2]. Mosquito senescence has been documented in *Aedes aegypti*, both under laboratory and semi-field conditions [36,37]. As pointed out by other authors [35,36,38-40], vector-borne disease models tend to dismiss evidence supporting age-dependent vector mortality [41] for the sake of tractability, and because of contradictory evidence between laboratory and field studies [2,5,42], often assuming a constant hazard (and hence an exponential distribution of survival times, shown to give a poor fit to our data) [43]. In addition, our data suggest that age-dependent mortality in the juvenile and adult stages of *Anopheles gambiae* s.s. mosquitoes may depend on environmental temperature.

The results presented here give a detailed picture of larval survival at a range of temperatures. Previous studies have examined the effect of temperature on larval mortality rates (19), the percentage of larvae surviving to adulthood [20,23], the combined effect of larval density and temperature on survival rates [44], the combined effect of inter-species competition and temperature on the proportion of larvae developing to adults [23], and the effect of altitudinal changes in temperature on the proportion of larvae developing to adults [14]. Our study is, to our knowledge, the first to follow *Anopheles gambiae* s.s. larvae during their entire lifecycle.

Previous research into adult mortality has examined the probability of daily survival within a range of temperatures from 5°C to 40°C and with a humidity range from 40% to 100% [17,18], the different mortalities of emerging males and females [33], the time to 50% survival at different temperatures [45,46], the proportion surviving after exposure to high temperatures [16], and survival at different combinations of temperature and relative humidity (RH) [29]. However, our study differs from these by allowing mosquitoes to blood-feed and oviposit, mimicking more closely their true fate as adults.

We informally compare our results on adult mortality at a larval temperature of 27°C and adult temperatures of 23°C, 27°C, and 31°C, with 75 ± 5% RH, with those reported by Bayoh and Lindsay [Unpublished pers. comm.] at 20°C, 25°C, and 30°C, with 80% RH, and find (by visual inspection) similar survival curves (Additional file 14: Figure S7). The increased mortality in our study is likely due to the difference in experimental protocol. Host-seeking, blood feeding and oviposition carry a fitness and survival cost, using metabolic vector resources (allocated to reproduction), incurring a risk of drowning while laying, and placing a stress associated with travelling and displacement [47]. Inter-study differences in malaria vector survival as measured in captivity can be, partly, associated with allowing or not further blood-meals and egg-laying events (Heather Ferguson, pers. comm.).

It appears that the degree of influence of *An. gambiae* larval temperature on adult survival is dependent on adult temperature itself. Further experimentation is needed to determine whether a threshold exists above which increasing larval temperature significantly reduces adult survival, or whether an increase in larval temperature of a certain magnitude will only affect adult survival within certain environmental temperature margins. However, these hypotheses do not take into account temperature fluctuations between day and night, or diurnal fluctuations more generally.

The final draft of the Fifth Assessment Report by the Intergovernmental Panel on Climate Change [48] for the

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near-term climate (2016–2035) suggests that the global mean surface air temperature will increase by approximately 0.3°C–0.7°C, and that there will be an increase in the duration, intensity, and spatial reach of heat-waves. In light of the results presented here, which indicate that small changes in temperature are less likely to affect survival than larger fluctuations, the predicted short-term changes in temperature may not strongly influence *An. gambiae s.s.* distribution in areas where this vector is already established and present. Air temperatures currently vary broadly across Africa, with night-time air temperatures ranging from 6°C to 29.5°C, and daytime air temperatures from 17°C to 41.3°C [49]. This implies that the sensitivity of *An. gambiae* s.s. to changes in environmental temperature will be extremely region-specific.

Our experimental design did not take into account temperature fluctuations or differences in humidity that would affect mosquito development and survival in the field. Further investigation is needed to examine the effects of other climatic and environmental factors on *An. gambiae* survival and development rates. More research is also needed into the influence of local air temperature fluctuations and how these affect the temperature of the water in mosquito breeding sites. In addition, *Anopheles gambiae* s.s. is only one of seven dominant vector species of human malaria on the African continent [50], and data regarding the sensitivity of these other species to temperature and other climate- and population-related factors are equally sparse, if not more so. Climate change is likely to influence the survival [51] and life-history parameters of different species of malaria vectors in different manners [52]. More extensive, species-specific data on the dependency of mosquito life-history parameters and population dynamics on climatic conditions, when coupled with geographically-detailed climate predictions, will enable more robust and reliable predictions of vector population dynamics and disease transmission.

Conclusions

Climate change is expected to lead to global and regional changes in environmental temperature and other climatic variables [48], which are likely to have an impact on vector distribution in sub-Saharan Africa and other malaria-prone regions [49,51]. It is thought that global warming may make currently inhospitable regions amenable to vector expansion along altitudinal gradients [1]. In order to generate useful predictions of malaria transmission and the impact of intervention programmes, the full impact of environmental conditions on the life-history parameters and population dynamics of disease vectors needs to be taken into account when forecasting transmission.

Our data show that the environmental temperatures to which *Anopheles gambiae s.s.* mosquitoes are exposed during both the juvenile and adult stages significantly affect the survival of this malaria vector both directly and indirectly, as temperatures during larval development influence adult survival. The direct effect of environmental temperature on larval and adult survival is highly significant for the range explored (23°C to 35°C), as it is for almost all temperature increases investigated.

We document here for the first time that the temperature to which *Anopheles gambiae* s.s. larvae are exposed during their development also influences the mortality of the adult females. This may have important implications for *Anopheles* population dynamics and ecology, and the diseases these mosquitoes transmit. Our results also show that the Gompertz distribution fits data on adult *Anopheles gambiae* survival in the laboratory significantly better than other parametric functions, including the exponential, implying that *Anopheles gambiae* mortality in the laboratory is age-dependent. This needs further confirmation from mortality data in the field, as evidence of age-dependent mortality has important implications for modelling vector population dynamics and the spread of malaria, requiring re-assessment of the common assumption in vector and transmission models that adult mosquito mortality does not depend on age.

Additional files

Additional file 1: Table S1. Akaike Information Criterion (AIC) values for the exponential, gamma, Gompertz, and Weibull fits to larval survival data (* indicates the best fit).

Additional file 2: Table S2. Akaike Information Criterion (AIC) values for the exponential, gamma, Gompertz, and Weibull fits to adult survival data (* indicates the best fit).

Additional file 3: Table S3. AICc values for the exponential, gamma, Gompertz, and Weibull fits to adult survival data, subdivided by larval temperature (* indicates the best fit, ‡ indicates where the Gompertz fit is not significantly worse than the best fit).

Additional file 4: Figure S1. Parametric fitting. An example of the fitting of four parametric survival functions (exponential (yellow), Gompertz (green), gamma (red), and Weibull (blue)) to larval survival data at environmental temperature 35°C.

Additional file 5: Table S4. Median survival times of *An. gambiae* s.s. larvae at different environmental temperatures. *ND: Not determined. Median survival defines the time point at which the survivorship curve crosses 0.5, or at which 50% of the sample is expected to survive. In this case, the survival function did not cross 0.5, and the median survival cannot be calculated.

Additional file 6: Table S5. Two-group comparisons and overall trend of the effect of larval environmental temperature on *An. gambiae* s.s. larval survival. *The comparison between 31°C and 23°C generated partly indistinguishable data, which did not allow us to perform a meaningful statistical test.

Additional file 7: Table S6. Median survival times of *An. gambiae* s.s. adults at different environmental temperatures. *ND: Not determined. Median survival defines the time point at which the survivorship curve crosses 0.5, or at which 50% of the sample is expected to survive. In this

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case, the survival function did not cross 0.5, and the median survival cannot be calculated.

Additional file 8: Figure S2. Gompertz fits to larval survival data. The Gompertz survival functions (red) are shown alongside the larval survival data at all environmental temperatures (23°C, 27°C, 31°C, and 35°C) to which they were fitted.

Additional file 9: Table S7. Two-group comparisons and overall trend of the effect of adult environmental temperature on *An. gambiae* s.s. adult survival.

Additional file 10: Figure S3. Gompertz fits to adult survival data. **(A)**. The Gompertz survival functions (red) are shown alongside the adult survival data at all adult temperatures (23°C, 27°C, 31°C) to which they were fitted. **(B)**. The Gompertz survival functions (red) are shown alongside the adult survival data at all combinations of larval and adult temperatures to which they were fitted.

Additional file 11: Figure S4. Values of the Gompertz survival function parameters, λ and *θ*. **(A)**. Parameters for the Gompertz survival function fitted to the larval survival data at each larval temperature are shown with their 95% confidence intervals (CI). **(B)**. Parameters for the Gompertz survival function fitted to the adult survival data at each adult temperature are shown with their 95% CI. (C). Parameters for the Gompertz survival function fitted to the adult survival data at each combination of larval and adult temperatures are shown with their 95% CI.

Additional file 12: Figure S5. Best-fit Gompertz survival function plotted against larval survival data. The Gompertz functions (blue) are shown alongside the larval survival data at all environmental temperatures (23°C, 27°C, 31°C, and 35°C).

Additional file 13: Figure S6. Gompertz survival function plotted alongside adult mortality data. **(A)**. The Gompertz functions (blue) are shown alongside the adult mortality data at all adult temperatures (23°C, 27°C, 31°C). **(B)**. The Gompertz functions (blue) are shown alongside the adult mortality data at all combinations of larval and adult temperatures.

Additional file 14: Figure S7. Comparison of survival curves with those generated by Bayoh and Lindsay (30). **(A)**. Survival curves by Bayoh and Lindsay, larval temperature 26°C, 80% RH. Adult survival at environmental temperature 20°C (yellow), 25°C (orange), and 30°C (red). **(B)**. Survival curves with data from this study, larval temperature 27°C, 75% RH. Adult survival at environmental temperature 23°C (yellow), 27°C (orange), 31°C (red).

Abbreviations

AIC: Akaike Information Criterion; AICc: Corrected Akaike Information Criterion; CI: Confidence Intervals; MLE: Maximum Likelihood Estimation; RH: Relative humidity; s.s.: Sensu stricto.

Competing interests

The authors declare that they have no competing interests.

Authors' contributions

CC-J, AS, PEP, JCK, and M-GB conceptualized the experimental design and study. CC-J carried out the experiments in JCK's laboratory, and analysed the data. CC-J, PEP, JCK, and M-GB wrote the first draft of the manuscript. All authors read and approved the final version of the manuscript.

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Article

Modelling *Anopheles gambiae s.s.* Population Dynamics with Temperature- and Age-Dependent Survival

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Abstract: Climate change and global warming are emerging as important threats to human health, particularly through the potential increase in vector- and water-borne diseases. Environmental variables are known to affect substantially the population dynamics and abundance of the poikilothermic vectors of disease, but the exact extent of this sensitivity is not well established. Focusing on malaria and its main vector in Africa, *Anopheles gambiae sensu stricto*, we present a set of novel mathematical models of climate-driven mosquito population dynamics motivated by experimental data suggesting that in *An. gambiae*, mortality is temperature and age dependent. We compared the performance of these models to that of a "standard" model ignoring age dependence. We used a longitudinal dataset of vector abundance over 36 months in sub-Saharan Africa for comparison between models that incorporate age dependence and one that does not, and observe that age-dependent models consistently fitted the data better than the reference model. This highlights that including age dependence in the vector component of mosquito-borne disease models may be important to predict more reliably disease transmission dynamics. Further data and

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studies are needed to enable improved fitting, leading to more accurate and informative model predictions for the *An. gambiae* malaria vector as well as for other disease vectors.

Keywords: mathematical modelling; climate change; mosquito population dynamics; *Anopheles gambiae s.s.*; senescence; malaria; vector-borne diseases

1. Introduction

Mathematical modelling is a useful tool for better understanding the epidemiology and transmission dynamics of infectious diseases, including vector-borne diseases (VBDs) such as malaria, and for better targeting and predicting the outcome of elimination efforts [1,2]. Mathematical models allow the integration of complex biological mechanisms (e.g., the lifecycle of the parasite and/or the vector's life history parameters), the details of which may not be known precisely, into one eloquent representation [3]. Models of VBDs must take into account, either explicitly or implicitly [4,5], the role played by the vectors in disease transmission. Where the vector component is modelled explicitly, it is important that it depicts the vector population dynamics as realistically as possible in the context of the overall biology and ecology of the vector [6–9].

For mosquito-borne diseases, the advantages of modelling the mosquito stages explicitly are immediately apparent for models aiming to assess the impact of larvicidal or pupacidal interventions [10,11], or for models wishing to ascertain the effect of external influences on each stage of mosquito development [12,13]. This is relevant, for example, when modelling the impact of climate change on VBDs. Climate change and global warming, and the accompanying changes in temperature, rainfall and humidity (leading to desiccation), as well as extreme events, are expected to influence considerably the spread of infectious diseases, particularly vector- and water-borne diseases [14–21]. The transmission of VBDs is sensitive to fluctuations in environmental variables, in large part due to the response of insect vectors to climatic factors: since insects are poikilothermic, their ecology, population dynamics, and spatial distribution depend strongly on weather-related phenomena [22–24].

As it has become more widely accepted that climate change and global warming may affect the spread of VBDs, including mosquito-borne diseases, by influencing vector ecology, experimental research has started to define the extent to which vectors' life-history parameters [25–29] and capacity to transmit diseases [29–32] depend on climatic variables such as temperature, rainfall and desiccation. Climate is expected particularly to influence vector survival [33,34], one of the key determinants of VBD transmission [35–38], in addition to development rates, reproduction, behaviour, and feeding patterns [33,39], all of which influence vector population dynamics, seasonal trends and geographic distribution. Given increasing evidence that weather-related factors affect vector life-history traits and hence disease transmission dynamics, it is important to incorporate the role of climatic factors when modelling vector populations and VBDs. Including a temperature component, in particular, is becoming increasingly common in models of vector abundance [9,40–44] and disease spread [45–47].

Faithfully modelling the biology and ecology of disease vectors relies upon the availability of comprehensive entomological data to inform model structure and parameterisation [7,48–50]. A lack of precise and adequate data may compromise or limit the accuracy and usefulness of the model output if it

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relies too heavily on the fitting of crucial and relevant parameters [51,52]. Data describing the different entomological processes contributing to vector population dynamics are, therefore, vital to ensure reliable model predictions. In the case of mosquito vectors, current research suggests there is some evidence that mortality is not only temperature dependent, but also age dependent [53–56]. However, this may only be evident in laboratory settings and it is uncertain whether wild mosquitoes may live long enough for age to become a significant cause of mortality, or indeed whether the reduction in survival due to environmental factors obscures the role of senescence in vector mortality [57,58]. Until recently, most models of VBDs have modelled constant, age-independent vector mortality, but it has been suggested that realistic age-dependent mortality rates may fit observed abundance data better [59–63]. Exactly how to include age-dependent death will depend on the vector species in question, as well as various other parameters connected with the biology of the vector, including feeding patterns and exposure to parasites, pathogens, and insecticides [64].

Despite the accumulation of experimental evidence from different species of disease vectors to support the inclusion of age-dependent survival, many VBD models still rely upon the framework of constant mortality rates, or mortality rates that depend solely on temperature. The study presented here is based upon recent laboratory work demonstrating that *Anopheles gambiae* sensu stricto (hereafter referred to simply as *Anopheles gambiae*), the primary vector of human malaria in Africa, does senesce, and that survival models incorporating age-dependent survival fit mortality data significantly better than those assuming constant, age-independent survival [34]. We present here a set of four mathematical models of *An. gambiae* population dynamics that enable us to test whether the age-dependent mortality observed in experimental data fits abundance data from sub-Saharan Africa better than the standard modelling paradigm of age-independent mortality rates. The models of age-dependent mortality we present here are based on this experimental data advocating that the survival of *Anopheles gambiae* depends on temperature and age, both in the larval and in the adult stages. We also incorporate other recent work on the influence of temperature on the fecundity and hatching rates of *Anopheles gambiae* [65].

This paper is organised in four sections: Section 2 develops the methodology behind model development, parameterisation, and fitting to the mosquito abundance data, as well as the criteria according to which relevant datasets were identified. Section 3 presents the results of a literature search to collate abundance data as well as the results of model fitting, the interpretation of the findings and a critique of our methods and findings. Finally, Section 4 details the key conclusions from this modelling study and places the importance of this work in a wider context.

2. Methods

2.1. Model Structures and Parameterisation

The lifecycle of the *Anopheles gambiae* mosquito comprises four main stages: eggs, larvae, pupae, and adults. The first three stages are aquatic, and only upon emergence into adults do mosquitoes leave the water. The larval and adult stages are the longest, with the larval phase consisting of four sub-stages (instars), while the egg and pupal stages are typically very short (depending on temperature). It is useful to model each stage explicitly, partly because the development parameters of each stage may vary considerably and the influence of external factors on these parameters may differ markedly, and partly

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because this enables the modelling of interventions targeted at specific stages (such as larval source management (LSM) for the juveniles or indoor residual spraying (IRS) for the adult females) [11,66]. In the models presented here, each stage of the mosquito lifecycle is modelled explicitly: the four larval instars are grouped into one larval stage (L) (due to a lack of instar-specific data), while the egg (E), pupal (P), and female adult (A) stages are all modelled independently. The four state variables ($E(t)$, $L(t)$, $P(t)$ and $A(t)$) track the number of mosquitoes within each stage at time t.

In order to test whether incorporating age-dependent mortality in *An. gambiae* population models translates into a better fit to observed abundance data, four models were developed. The first model represents a baseline model for comparison, with exponentially-distributed survival times for egg, larval, pupal, and adult stages, and where stage-specific mortality depends only on temperature. In the second model, larval mortality is assumed to be temperature and age dependent, while in the third, adult mortality is modelled as temperature and age dependent. The final model combines age-dependent larval mortality (as in model 2) with age-dependent adult mortality (as in model 3). Egg and pupal mortality is assumed to be age independent in all four models, on the basis that mosquitoes remain in these stages for too short a time to be affected by senescence (and a lack of experimental data to test for evidence to the contrary). The effect of relative humidity (RH) on the survival of *An. gambiae* is not explicitly modelled here, even though there is some evidence that low levels of humidity may influence adult mosquito longevity [67,68].

In the following, we describe sequentially the models developed, with Tables 1 and 2 defining the notation used for model parameters, their values and units, and their sources. Model 1 represents a baseline model (Figure 1 and Equation (1)) that represents survival during the larval and adult stages as dependent solely on temperature, thus assuming that the time spent in each stage is exponentially distributed.

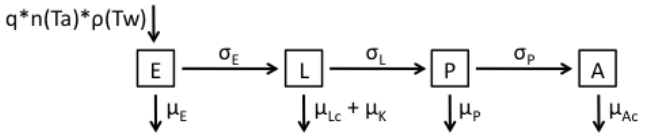


Figure 1. Flow diagram representing the structure of Model 1.

$$\begin{aligned}
\frac{dE}{dt} &= qn(Ta)\rho(Tw)A-(\mu_E+\sigma_E)E,\\
\frac{dL}{dt} &= \sigma_E E-(\mu_{Lc}+\mu_K+\sigma_L)L,\\
\frac{dP}{dt} &= \sigma_L L-(\mu_P+\sigma_P)P,\\
\frac{dA}{dt} &= \frac{\sigma_P P}{2}-\mu_{Ac}A.
\end{aligned} \tag{1}$$

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Table 1. Average duration (in days) of egg, larval, and pupal stages at water temperature Tw according to Parham *et al*. [69], with corrected coefficients.

Parameter	Functional Form for Average Stage Duration
d_E (eggs)	$\left(1.011+\frac{20.212}{1+\left(Tw/12.096\right)^{4.839}}\right)$
d_L (larvae)	$\left(8.13+\frac{13.794}{1+\left(Tw/20.742\right)^{8.946}}\right)-\left(1.011+\frac{20.212}{1+\left(Tw/12.096\right)^{4.839}}\right)$
d_P (pupae)	$\left(8.56+\frac{20.654}{1+\left(Tw/19.759\right)^{6.827}}\right)-\left(8.13+\frac{13.794}{1+\left(Tw/20.742\right)^{8.946}}\right)-\left(1.011+\frac{20.212}{1+\left(Tw/12.096\right)^{4.839}}\right)$

Table 2. Model parameters and parameter values. Parameters marked * were inferred as described below.

Parameter	Definition	Unit	Prior	Posterior
q	Proportion of adult females laying eggs	−	0.61 − 0.85 [65]	*
$n(T_a)$	Number of eggs laid per female	−	$-1.1057\ Ta^2 + 56.208\ Ta - 662.1$ [65]	
$\rho(T_w)$	Proportion of eggs hatching	−	$-0.0034\ Tw^2 + 0.1719\ Tw - 1.248$ [65]	
μ_E	Per capita egg mortality rate	days^{-1}	0.32 − 0.8 [10,70]	*
μ_L	Per capita age-dependent larval mortality rate	days^{-1}	$1/\alpha_L\beta_L$	
μ_{LC}	Per capita age-independent larval mortality rate	days^{-1}	$0.0013\ Tw^2 - 0.0704\ Tw + 0.9581$	
μ_P	Per capita pupal mortality rate	days^{-1}	0.25 [11]	
μ_A	Per capita age-dependent adult mortality rate	days^{-1}	$1/\alpha_A\beta_A$	
μ_{AC}	Per capita age-independent adult mortality rate	days^{-1}	$(5.37\times10^{-5})\ e^{0.228Ta}$	
μ_K	Per capita density- (and rainfall-) dependent larval mortality rate	days^{-1}	$\mu_C \sum L_i / K$ [11]	

Table 2. *Cont*.

Parameter	Definition	Unit	Prior	Posterior
μ_C	Constant	days^{-1}	0–10,000	*
K	Carrying capacity	−	[11]	
τ	Days of rainfall contributing to carrying capacity	days	<10 [11]	*
α_L	Shape parameter of larval gamma hazard function	−	7	
β_L	Scale parameter of larval gamma hazard function	days	$-0.0112\ Tw^2 + 6.0775\ Tw - 6.709$	
α_A	Shape parameter of adult gamma hazard function	−	3	
β_A	Scale parameter of adult gamma hazard function	days	$171.26\ e^{-0.1191Ta}$	
σ_E	Per capita egg development rate	days^{-1}	$1/d_E$ [68]	
σ_L	Per capita larval development rate	days^{-1}	$1/d_L$ [68]	
σ_P	Per capita pupal development rate	days^{-1}	$1/d_P$ [68]	
σ_A	Per capita adult development rate	days^{-1}	$1/d_A$ [68]	
Δ_T	Difference between environmental air and water temperature	°C	2.9 − 7.6 [71]	*

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The second model (Figure 2 and Equation (2)) takes into account age-dependent mortality affecting larvae via a gamma distribution with parameters α_L and β_L by subdividing the larval stage into α_L subclasses (the number of sub-classes is determined as defined below) [72,73]. The rate at which larvae progress through the subclasses is set as $\alpha_L\mu_L$, where μ_L is equal to $1/\alpha_L\beta_L$. Upon hatching, eggs will enter the first larval subclass (L_1), in which they either progress to the next subclass (L_2) at temperature-dependent rate 7 μ_L, progress to pupae at temperature-dependent rate σ_L, or die due to overcrowding at density-dependent rate μ_K. This process continues as they progress through all subsequent subclasses.

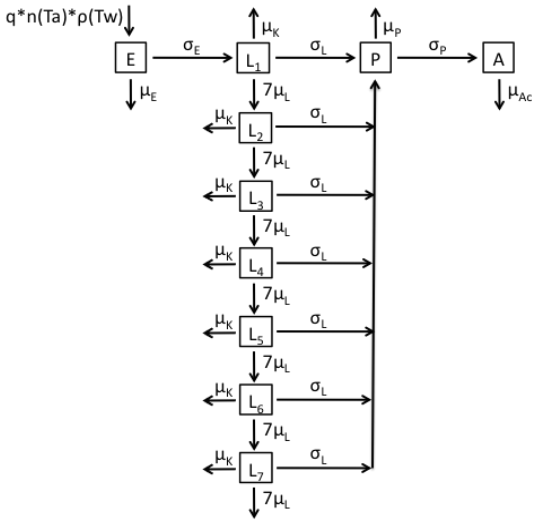


Figure 2. Flow diagram representing the structure of Model 2.

$$\begin{aligned}
\frac{dE}{dt} &= qn(Ta)\rho(Tw)A-(\mu_E+\sigma_E)E,\\
\frac{dL_i}{dt} &= \begin{cases}\sigma_E E-(7\mu_L+\mu_K+\sigma_L)L_i & , \quad i=1\\ 7\mu_L L_{i-1}-(7\mu_L+\mu_K+\sigma_L)L_i & , \quad 2\le i\le 7\end{cases}\\
\frac{dP}{dt} &= \sigma_L\sum_{i=1}^{7}L_i-(\mu_P+\sigma_P)P,\\
\frac{dA}{dt} &= \frac{\sigma_P P}{2}-\mu_{Ac}A.
\end{aligned} \tag{2}$$

The third model (Figure 3 and Equation (3)) retains the temperature- and age-independent larval mortality of the first model, but now models age-dependent adult mortality. This is modelled in a similar fashion to the age-dependent larval mortality in model 2, namely using a gamma distribution with parameters α_A and β_A. The adult stage of the model comprises α_A subclasses (the number of subclasses is determined as defined below), and adults progress through the subclasses at daily temperature-dependent rate $\alpha_A\mu_A$, with μ_A equal to $1/\alpha_A\beta_A$. Upon entering the first adult subclass (A_1), female mosquitoes

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sequentially progress through adult subclasses at rate 3 μ_A, until they drop out of the model. The number of eggs laid in this model is dependent on the total number of adult females in all three adult subclasses.

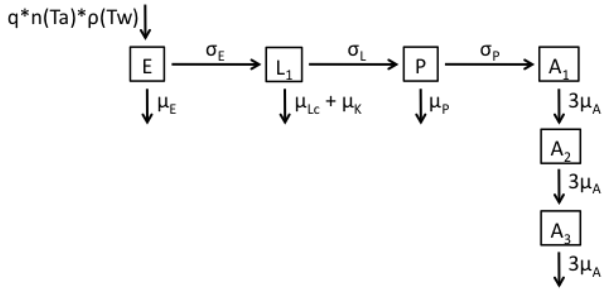


Figure 3. Flow diagram representing the structure of Model 3.

$$\begin{aligned}
\frac{dE}{dt} &= qn(Ta)\rho(Tw)\sum_{i=1}^{3} A_i - (\mu_E + \sigma_E)E, \\
\frac{dL}{dt} &= \sigma_E E - (\mu_{Lc} + \mu_K + \sigma_L)L, \\
\frac{dP}{dt} &= \sigma_L L - (\mu_P + \sigma_P)P, \\
\frac{dA_i}{dt} &= \begin{cases} \frac{\sigma_P P}{2} - 3\mu_A A_i & , \quad i = 1 \\ 3\mu_A A_{i-1} - 3\mu_A A_i & , \quad i = 2,3. \end{cases}
\end{aligned} \tag{3}$$

The fourth model (Figure 4 and Equation (4)) is a combination of models 2 and 3, comprising temperature- and age-dependent survival in both the larval and adult stages. The number of eggs laid and the number of pupae developing from larvae are also calculated according to the respective appropriate models.

$$\begin{aligned}
\frac{dE}{dt} &= qn(Ta)\rho(Tw)\sum_{i=1}^{7} A_i - (\mu_E + \sigma_E)E, \\
\frac{dL_i}{dt} &= \begin{cases} \sigma_E E - (7\mu_L + \mu_K + \sigma_L)L_i & , \quad i = 1 \\ 7\mu_L L_{i-1} - (7\mu_L + \mu_K + \sigma_L)L_i & , \quad 2 \le i \le 7 \end{cases} \\
\frac{dP}{dt} &= \sigma_L \sum_{i=1}^{7} L_i - (\mu_P + \sigma_P)P, \\
\frac{dA_i}{dt} &= \begin{cases} \frac{\sigma_P P}{2} - 3\mu_A A_i & , \quad i = 1 \\ 3\mu_A A_{i-1} - 3\mu_A A_i & , \quad i = 2,3. \end{cases}
\end{aligned} \tag{4}$$

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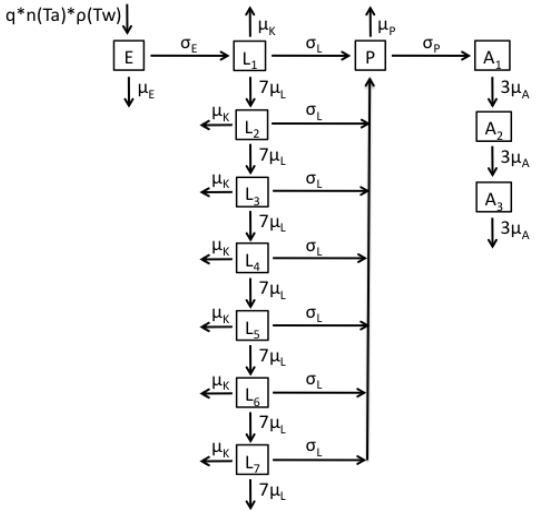


Figure 4. Flow diagram representing the structure of Model 4.

On average, a proportion q of female adults will lay a number of eggs $n(T_a)$, a proportion $\rho(T_w)$ of which will hatch [65] (where the former depends on the environmental (air) temperature of the adults T_a, and the latter depends on the water temperature T_w in which the eggs are laid. Eggs undergo a fixed, temperature-independent daily mortality at rate μ_E, or progress to larvae at a daily rate σ_E, which is given by the inverse of the duration of the egg stage $1/d_E$ (Table 1) [69] as defined by Bayoh and Lindsay (unpublished data).

Larvae either undergo a temperature-dependent, but age-independent, daily mortality at rate μ_{Lc} in models 1 and 3, or a temperature- and age-dependent daily mortality at rate μ_L in models 2 and 4. In addition, larvae are subjected to density-dependent regulation, which is represented by an additional daily mortality rate μ_K. Larvae that do not die progress to pupae at rate σ_E, which is given by the inverse of the duration of the larval stage (Table 1) [69]. Pupae either die at a fixed, temperature-independent daily mortality rate μ_P or progress to adults at rate σ_P, which is given by the inverse of the duration of the pupal stage (Table 1) [69]. Only adult females are explicitly modelled and it is assumed that half of all pupae developing into adults are females [74]. Adults either die at a temperature-dependent, but age-independent, daily mortality rate μ_{Ac} in models 1 and 2, or at a temperature- and age-dependent daily mortality rate μ_A, in models 3 and 4. The values of fixed parameters in the models are given in Table 2 together with the references of provenance. In the case of the parameters estimated by fitting the models to data (using Bayesian statistics), the prior values are those used as initial values (informed by the literature where available). The asterisks indicate those values that will be obtained from the posterior distribution.

Given experimental evidence that mosquitoes senesce [61,62], the common assumption that their mortality rates are constant may be oversimplified, as it does not take into account that as mosquitoes get older and undergo multiple gonotrophic cycles, they are more likely to die. This is supported by laboratory evidence that survival times are not exponentially distributed, and therefore a constant hazard

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may not be truly representative of the *An. gambiae* survival function $S(t)$ [34]. Christiansen-Jucht *et al.* [34] fitted four different parametric forms for $S(t)$ (exponential, gamma, Weibull and Gompertz) by Maximum Likelihood Estimation (MLE) to laboratory survival data at four different temperatures (23 °C, 27 °C, 31 °C, and 35 °C), and compared the fits using Akaike Information Criterion (AIC). At each temperature, and for both larval and adult stages, the exponential distribution was found to fit the data significantly worse than all other parametric forms, indicating that age-independent survival models are not appropriate to describe *An. gambiae* survival. Although it was shown that the Gompertz distribution fits the experimental survival data best, the gamma function was chosen here since (a) it also fitted the data significantly better than the exponential distribution at all temperatures, and was not significantly worse than the Gompertz in the majority of cases according to the AIC [34]; and (b) the gamma distribution is mathematically and computationally convenient since it can be decomposed into multiple exponentially-distributed models [72].

A constant (time-independent) hazard rate, derived from the exponential model fit of $S(t)$ to the survival data in [34], was fitted to the laboratory mortality data (Figures 5 and 6, blue lines). In addition, the temperature dependence of the hazard functions μ_{Lc} and μ_{Ac} was plotted and a functional form fitted (Figure 7 and Table 2) to obtain the larval and adult age-independent mortality rates (μ_{Lc} and μ_{Ac} respectively). In order to model more realistically age-dependent mortality, the gamma distribution was fitted to the laboratory survival data in [34] (Figures 5 and 6, red lines).

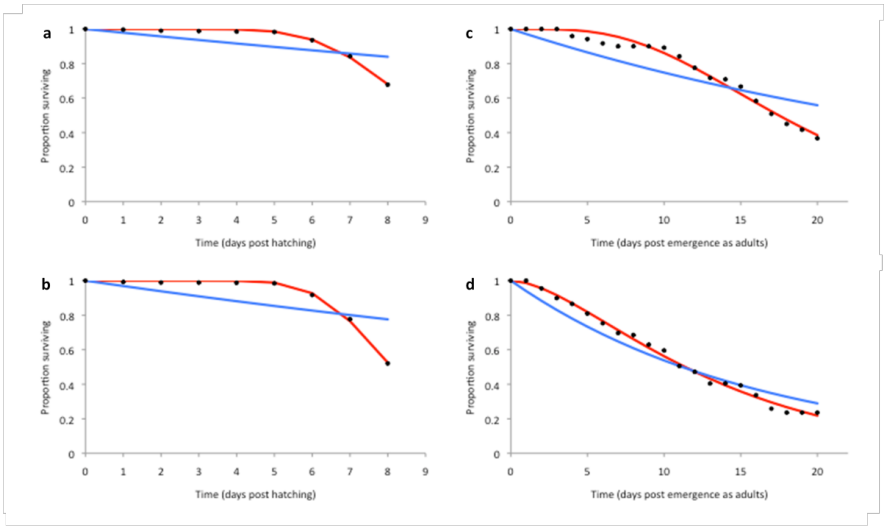


Figure 5. Fit of exponential (blue lines) and gamma (red lines) survival functions $S(t)$ to the laboratory survival data in [34] at 27 °C (**a**,**c**) and 31 °C (**b**,**d**). Here, (**a**,**b**) are for larvae, while (**c**,**d**) are for adults.

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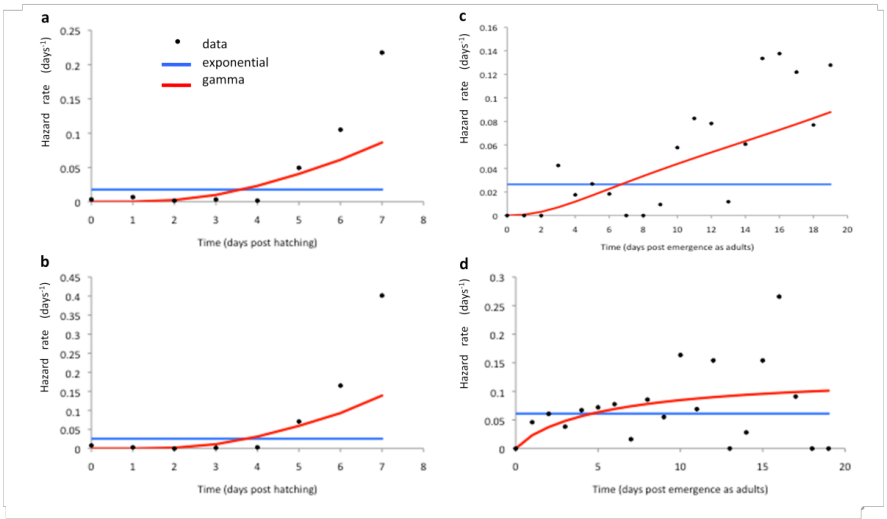


Figure 6. Fit of constant- (blue lines) and time (age) dependent (red lines) hazard functions to the laboratory mortality data in [34] at 27 °C (**a**,**c**) and 31 °C (**b**,**d**). Here, (**a**,**b**) are for larvae, while (**c**,**d**) are for adults. The constant hazard corresponds to the exponential model, whilst the age-dependent hazard corresponds to the gamma distribution of survival times.

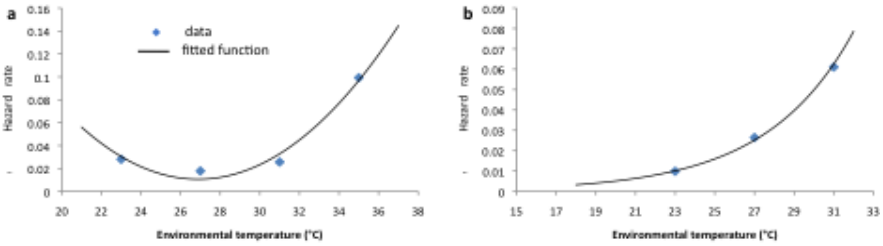


Figure 7. Age-independent larval (**a**) and adult (**b**) mortality rate as a function of environmental temperature (with the best-fit functional forms given in Table 2).

The gamma distribution is a two-parameter probability distribution; here, both parameters vary with temperature. Since the shape parameter (here, α) corresponds to the number of subclasses into which the relevant stage (larvae or adults) is divided [72,73], α had to be assigned a fixed value independent of temperature. To determine the integer value of α that yielded the closest fit to the MLE-defined best-fit α at the larval and adult stages, we assigned α integer values either side of the original values of α obtained by MLE.

The scale parameter β of the relevant gamma distribution was then obtained by fixing the value of α and fitting β; the goodness-of-fit of the resulting gamma distribution was compared to the original best-fit distribution using AIC. The integer value of α for which the sum of the differences between AIC across all larval or adult temperatures was minimised was chosen as the fixed value of α for that stage (Supplementary Tables S1 and S2). For the larval and adult stages, α was set to seven and three

respectively (this is further explained below). The gamma distribution was then fitted by MLE to the survival data with β allowed to vary freely until the best fit was obtained for fixed α.

The integer values of α and the original best-fit MLE-determined values of α at different temperatures are shown in Supplementary Tables S1 (for the larval stage) and S2 (for the adult stage), along with the difference between the AIC value of the best fit and the AIC values of the different fits for fixed α. The MLE-fitted values of β (for fixed α) were plotted and a functional form fitted to determine how β varies with temperature for each stage (see Figure 8; the fitted functional forms are given in Table 2). To model gamma-distributed survival, while still maintaining a fixed average life expectancy that depends on temperature only, the rate of progression between sub-stages was defined as $\alpha_L\mu_L$ for larvae and $\alpha_A\mu_A$ for adults, where $\mu_L = 1/\alpha_L\beta_L$ and $\mu_A = 1/\alpha_A\beta_A$. This ensures that the average life expectancy of larvae and adults is still $1/\beta_L$ and $1/\beta_A$ respectively. As described above, $\alpha_L = 7$ and $\alpha_A = 3$, and both β_L and β_A depend on temperature.

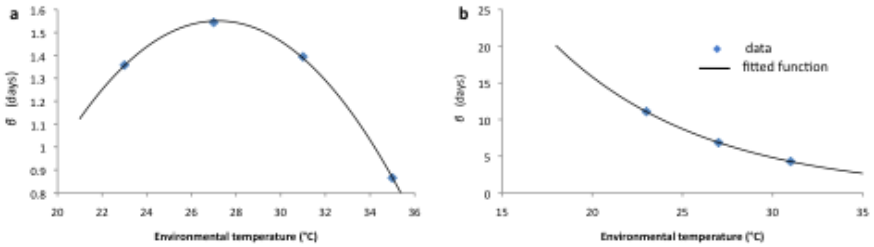


Figure 8. Value of β for the gamma larval (**a**) and adult (**b**) hazard rate as a function of environmental temperature.

In the models presented here, density dependence is only applied to the larval stage, as the mosquitoes are assumed to spend too little time, by comparison, in the egg and pupal stages for density to be a significant limiting factor [75,76]. The additional hazard on larvae to account for density-dependent mortality (μ_K) was derived from White *et al*. [11] as,

$$\mu_{K(t)} = \mu_C \left(\frac{\sum_{i=1}^{7} L_i}{K(t)} \right), \tag{5}$$

where $K(t)$ is the environmental carrying capacity at time t, and μ_C, which quantifies the magnitude of the density dependence, is a free parameter to be optimised in the model fitting. This formulation assumes the density-dependent mortality rate to be linearly proportional to the total number of mosquitoes in the larval stages. Here, $K(t)$ is based on the form that was found to fit best the model of [11] to the Garki dataset [77], namely,

$$K(t) = \frac{1}{\tau\left(1 - e^{-t/\tau}\right)} \int_0^t e^{-(t-t')/\tau} rain(t')dt'. \tag{6}$$

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2.2. Longitudinal Data for Model Fitting

A systematic literature review was carried out across the databases PubMed, Web of Knowledge, Google Scholar, and Ovid SP, and all articles published up until 14 February 2015 were considered. Primary key terms *Anopheles*, *gambiae*, *Africa*, *long-term*, *longitudinal*, and *temporal*, and secondary terms *abundance*, *abundance data*, *population*, *density*, *population density*, *mosquitoes*, *monthly*, *weekly*, and *daily* were used in combination with Boolean operators to direct searches. The titles and abstracts of resulting searches were screened for their likelihood to contain data on the collection of *An. gambiae* mosquitoes in any African country over a minimum continuous period of 12 months.

If the initial requirements were met, the full article was retrieved and detailed inclusion and exclusion criteria applied. *An. gambiae s.s.* was the primary focus of data collation, but articles with data on *An. gambiae sensu lato* were also accepted. Records of abundance data collected monthly, weekly, or daily were included provided studies met the requirement of a minimum collection period of 12 months between 1 January 2000 and 31 December 2009. Studies that reported only annual abundance data were excluded. Abundance data for all life-history stages (eggs, larvae, pupae, or adults) were accepted, although the majority of articles reported adult mosquito counts exclusively. Only studies reporting on the natural population dynamics of *An. gambiae* in the wild were included: abundance data collected during implementation of vector control intervention programmes, or from studies that chemically or genetically modified mosquito populations were excluded. Household collection data were accepted only if abundance data were from outdoor collections, and available for all households within the geographical region of the study. The bibliography list of articles meeting all criteria was also examined for additional relevant references.

Where raw data were available, mosquito numbers were lifted directly from the article, while data presented only in figures were extracted using GraphClick, Version 3.0.2 (Arizona Software, 2010). For articles in which abundance data were mentioned (or referred to) but not explicitly given, or where the data could not be recorded, the study authors were contacted directly to request the raw data. When authors did not respond, or if the data did not meet the inclusion criteria, the article was excluded. Where geographical coordinates were explicitly given, the city and country were entered into [78] to retrieve latitude and longitude. Locations were mapped using BatchGeo [79].

2.3. Model Fitting

Since the datasets to which model fitting was undertaken reported only the number of mosquitoes caught per month, we assumed that the data (D) are independently normally distributed, with a mean equal to the expected average monthly vector abundance for month i(δ_i) and a standard deviation σ_i equal to the maximum value of all observations for month i. For months with low or no mosquito counts, a minimum standard deviation of 0.1 was assumed. The probability of obtaining the data (D), given a model M and a set of parameters θ is given by:

$$P(D|\theta,M)=\prod_i \frac{1}{\sigma\sqrt{2\pi}} e^{-\frac{1}{2}\left(\frac{\delta_i - n_F y_i(\theta,M)}{\sigma}\right)^2} \quad (7)$$

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where n_F is a normalisation factor applied to the simulated data given model M and parameters θ ($y_i(\theta, M)$) and accounts for any systematic differences between observed abundance and model simulations. This normalisation factor also allows for adjustment between the models, which track the number of adult females, and the data, which include the abundance of male and female adults. Mean monthly mosquito numbers were calculated by averaging across daily model simulation results. All initial conditions were set to unity, and simulations were started six months prior to the date of the first observed data point to reduce the impact of the initial conditions (and model transients) on model fitting.

Assuming a uniform prior for all inferred parameters (marked * in Table 2) within the boundaries specified in Table 2, the posterior probability (the probability of observing θ given D and M) is defined as,

$$P(\theta|D,M) \propto P(D|\theta,M). \tag{8}$$

For each model, we arrived at a set of parameters maximising the posterior distribution, the maximum *a posteriori* (MAP) estimate, through multiple iterations of the basin-hopping algorithm implemented using the in-built SciPy package in Python (v. 2.7). The Bayesian Information Criterion (*BIC*) was calculated to enable inter-model comparisons as,

$$BIC = -2\ln\hat{\theta} + k\ln n, \tag{9}$$

where $\hat{\theta}$ is the MAP estimate, k is the number of inferred parameters, and n is the number of data points [80]. The commonly used rule of thumb is that models with a difference of ≤2 in BIC values are more or less indistinguishable, and models with a difference of >4 in BIC values are clearly distinguishable [81,82].

We also calculated model probabilities given the posterior distribution around $\hat{\theta}$ Bayesian model selection [83] defines the probability of a model given data, D, as,

$$P(M|D) \propto P(D|M)P(M) = P(M)\int P(D|\theta,M)P(\theta|M)d\theta, \tag{10}$$

where $P(M)$ is the prior probability of model M and $P(\theta/M)$ is the prior probability of parameter set θ. Assuming a uniform distribution for the models and parameters, we have,

$$P(M|D) \propto \int P(D|\theta,M)d\theta. \tag{11}$$

By replacing $P(D/\theta, M)$ in (11) by its expression in Equation (7), we obtain

$$P(M|D) \propto \int e^{-\frac{1}{2}\left(\frac{\delta_i - n_F y_i(\theta,M)}{\sigma}\right)^2} d\theta. \tag{12}$$

The integral was calculated via Monte Carlo integration with samples from the posterior distribution around $\hat{\theta}$, which were obtained using an adaptive Metropolis-Hastings algorithm [84] with initial conditions in the locality of $\hat{\theta}$. For the sake of simplicity, we assumed that the main contributor to the model probability was the basin of the global optimum represented by $\hat{\theta}$ and discarded the alternative less probable local optima.

For each model, parameter sensitivities explored the impact of simultaneous parameter variations, and were calculated using samples from the posterior distribution around $\hat{\theta}$. We approximated the

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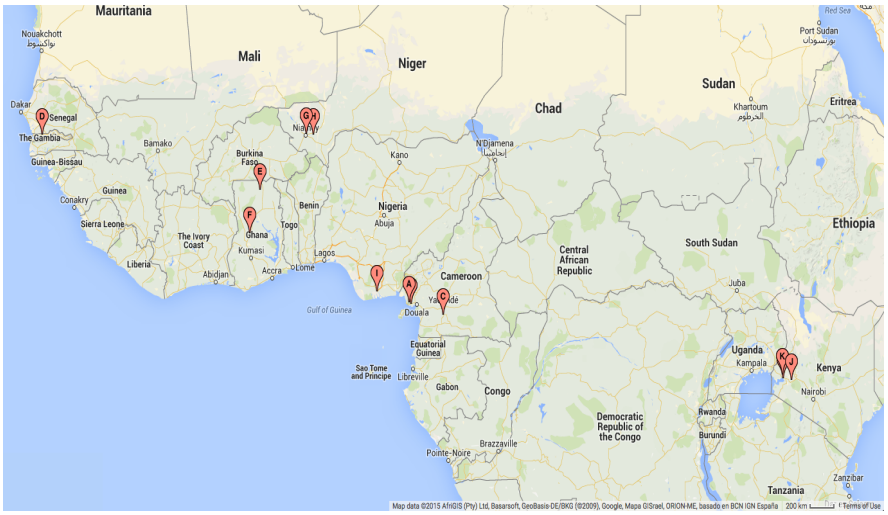


Figure 10. Map of geographical locations of datasets to which the models were fitted.

Table 3. Longitudinal datasets of *An. gambiae* abundance.

Map Ref	Geographical Location	Study Dates	Study Duration (Months)	Mosquito Species and Stage
A	Likoko, Cameroon	October 2002–September 2003	12	*An. gambiae s.l.* adults
B	Mutengene, Cameroon [91]	October 2004–September 2005	12	*An. gambiae s.s.* adults
C	Ekombitié, Cameroon [92]	January 2007–December 2007	12	*An. gambiae s.l.* adults
D	Njabakunda, The Gambia [93]	April 2007–March 2009	24	*An. gambiae s.s.* adults
E	Kassena, Ghana [94]	November 2001–October 2004	36	*An. gambiae s.s.* adults
F	Kintampo, Ghana [95]	November 2003–November 2005	25	*An. gambiae s.s.* adults
G	Banizoumbou, Niger [96]	May 2005–December 2006	20	*An. gambiae s.l.* adults
H	Zindarou, Niger [97]	July 2005–December 2006	18	*An. gambiae s.l.* adults
I	Ogbakiri, Nigeria [98]	September 2005–August 2006	12	*An. gambiae s.l.* adults
J	Fort Ternan, Kenya [99]			
K	Lunyerere, Kenya [99]	March 2006–March 2008	25	*An. gambiae s.l.* larvae
L	Nyalenda, Kenya [99]			

Our systematic review indicates that while longitudinal *An. gambiae* abundance data exist for several locations across sub-Saharan Africa at different climates, altitudes, and within different environments, these vary widely in terms of duration, ease of availability, provision of sufficient detail to enable robust model fitting, and overall data quality. Since the main purpose of this review was to assemble a collection of datasets that could be used to inform model fitting, it is clear that longer time-series would enable better model fitting to temporal trends in the data. We found that only half of the datasets obtained described monthly-resolution mosquito abundance for a period longer than 2 years (which would facilitate consistent vector behaviour and climate-driven population response to emerge as distinct temporal patterns, and thus would increase the likelihood of a good fit to the data [100]).

The quality of these datasets, and the level of detail with which abundance data are recorded and/or made available, strongly influenced the ability to fit the four models to these abundance datasets,

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and, hence, the accuracy of the models' predictions. Although 12 datasets matched our inclusion criteria, the methods sections in their respective publications almost always reported only the number of mosquitoes caught per month, with very little (or no) information on the number of catches or the frequency of catches within a month. This unavoidably results in model fitting to these datasets requiring *a priori* assumptions in the likelihood expression about the mean and standard deviation around the total monthly number of catches. Here, since the strength (and likely effect) of these assumptions increases for datasets reporting shorter time-series, we fit our four models only to the longest dataset [94].

3.2. Model Fitting

Figure 11 compares the performance of models 1 to 4 (under their respective best-fit parameters shown in Table 4) with the abundance data reported in [94], and Table 5 gives the BIC values for each model and Pearson's correlation coefficients (r) for the correlation between each model and the data. All four models were consistent in predicting very similar values for the proportion of adult females that lay eggs, and for the daily egg mortality rate. In both cases, the predicted values fell well within the prior ranges predicted by experimental data [10,65,70]. Three of the models, including the two best fitting models (3 and 4), predict a difference between environmental air and water temperature of approximately 7 °C, which is consistent with the higher end of the range observed in experimental data [71], and suggests that models assuming no difference between air and water temperature may not realistically capture the full effect of temperature on survival, likely leading to an underestimate of the influence of temperature on the mortality of the aquatic stages. All models predict very small values for weighting factor on the number of rainfall days τ contributing to breeding site carrying capacity (and therefore to density-dependent mortality of the larval stages), although the optimised values of τ given by models 3 and 4 are considerably smaller than those of models 1 and 2. In all cases, this value corresponded to less than one day, which indicates that, for this dataset, the cumulative effects of rainfall are weighted almost equally.

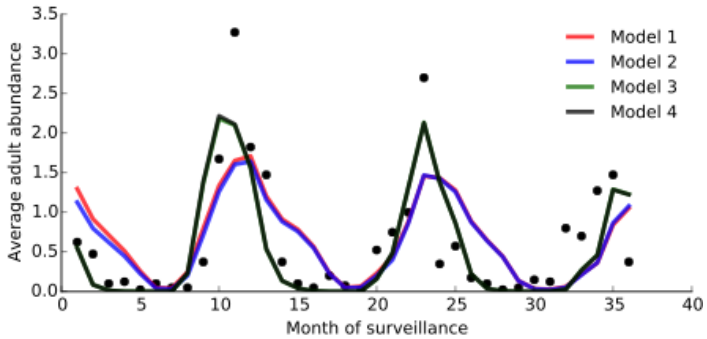


Figure 11. Agreement between data (solid markers) and model predictions (solid lines). Black dots represent the average adult vector counts for each month from November 2001 to November 2004 from [94]. Solid lines show the best-fitting model predictions. Models 1–4 are represented by red, blue, green, and grey lines, respectively.

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Table 4. Fitting-inferred parameter values. Parameters q, μ_E, μ_C, τ, and ΔT are described in Table 2, and n_F is the normalisation factor applied to the simulated data.

Model	Parameters					
	n_F	q	μ_E	μ_C	τ	ΔT
1	0.466	0.636	0.481	266.915	0.472	3.066
2	0.026	0.613	0.485	16.153	0.689	7.165
3	34.308	0.72	0.501	5886.43	0.087	7.196
4	11.453	0.656	0.533	1753.52	0.095	7.092

Table 5. Bayesian Information Criterion (BIC) values for each model, and Pearson correlation coefficient (r) values describing the models' fits to data from [94].

Variables	Model 1	Model 2	Model 3	Model 4
BIC	174.22	174.18	173.82	173.82
r	0.65	0.66	0.81	0.81

The fitted population dynamics and performance of Models 1 and 2 are very similar, and Models 3 and 4 almost identical. The models incorporating adult-only age-dependent mortality (Model 3) and adult and larval age-dependent mortality (Model 4) fit the data better than the "baseline" model with no age-dependent mortality in any stage (Model 1) and with larval-only age-dependent mortality (Model 2). While the difference in BIC values between Models 1 and 2, and Models 3 and 4, is less than 2, information criteria do not provide a measure of statistical significance: models with a lower BIC fit the data better, but there is no measure of how much better they fit [82]. However, the Pearson correlation coefficients show that Models 3 and 4 fit the data considerably better than models 1 and 2, and this is consistent with the 95% credible intervals (CI) shown in Figure 12. It is clear from Figure 12 that the fit of Model 1 is considerably worse than all other models at reliably capturing the main data peaks and troughs, as these points generally lie outside the 95% CI. Model 2 captures the troughs much better than the first model, but the 95% CI still fail to include the extreme peaks in mosquito abundance. The fits of Models 3 and 4 are both much closer to the data and the 95% CI in both models generally capture well both the peaks and troughs in the time-series.

It is evident from the BIC values and correlation coefficients that Models 2 and 4, which include age-dependent mortality in the larval stage, yield no improvement on the fitting compared, respectively, with Models 1 (temperature-dependent mortality only) and 3 (age-dependent mortality in the adult stages only). This suggests that including age-dependent mortality in the larval stages merely serves to increase the complexity and dimensionality of the models, but without contributing to overall model performance or predictive ability. Given the desire, wherever possible, for parsimony in model construction, including age-dependent survival in the adult stages when modelling *An. gambiae* population dynamics appears to be an important requirement for developing more realistic models, whereas including age-dependent survival in the larval stages does not improve model predictions. Given this result, and the fact that age-dependent mortality has been reported in other mosquito species [53,61], it may prove equally important to include senescence in models of other mosquito population dynamics and

mosquito-borne diseases. However, the availability of extensive and good quality experimental laboratory and field data on the effects of temperature and age on survival will be necessary to inform such models.

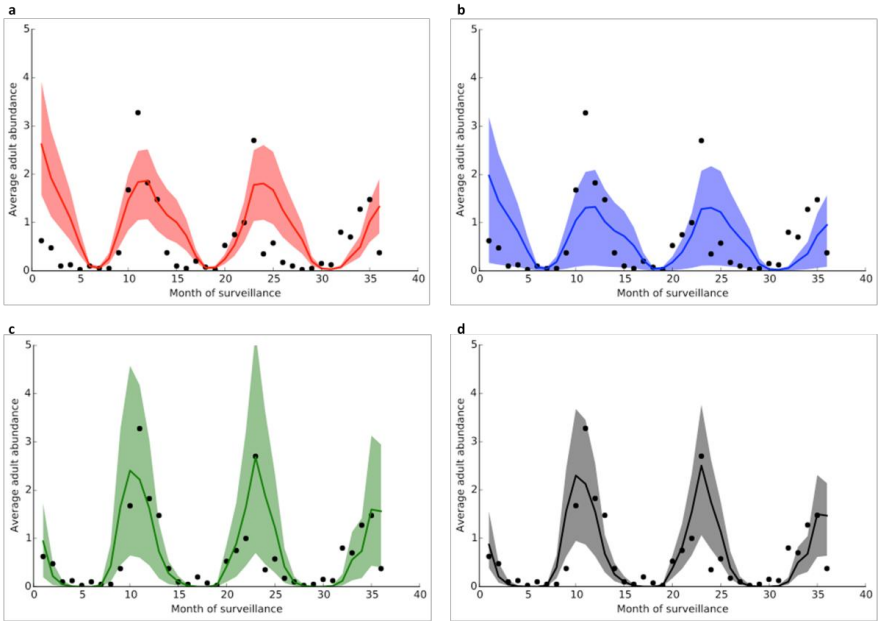


Figure 12. Model predictions (solid colour lines) and 95% Bayesian credible intervals (colour shaded areas) for model 1 (**a**), model 2 (**b**), model 3 (**c**), and model 4 (**d**); black dots are the data and the colour legend is the same as in Figure 11.

Sensitivity analysis of each model to the six fitted parameters (Supplementary Table S3) indicates that all four models are most sensitive to variations in the proportion of adult females laying eggs (q), as well as, to a slightly lesser extent, changes in the daily mortality rate of eggs (μ_E). This is to be expected, as these parameters are crucial in the reproduction, fitness, and survival of the mosquito, and serves to highlight on which entomological parameters we need more detailed and precise data in order to produce more reliable and robust models of disease vectors. All the models are also very sensitive to the difference between environmental air and water temperatures (ΔT), which further emphasises the importance of modelling the higher temperatures of the water bodies in which the immature stages of the mosquito develop, and suggests that models assuming water temperatures are the same as air temperatures may be missing out on a subtle determinant of mortality. This also highlights the necessity of measuring water temperature specifically in experimental work that aims to define the effect of temperature on the survival of *An. gambiae* aquatic stages, although this observation is likely applicable to other mosquito disease vectors also. The sensitivity of all models to variations in the difference between air and water temperature also emphasise that the population dynamics of *An. gambiae* are dependent not just on mean environmental temperatures, but also on small temperature fluctuations, which is consistent with previous experimental and theoretical work [9,25].

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While models 1, 2, and 4 were robust to variations in the other three inferred parameters, model 3 displayed a greater sensitivity to changes in the values of μ_C, suggesting that mosquito abundance is more sensitive to density-dependent mortality in the larval stages when age-dependent mortality is taken into account in the adult stages only.

In the context of the modelling study undertaken here, two key types of uncertainty should be assessed, namely parameter uncertainty and uncertainty resulting from model structure [101–103]. In terms of the latter, we decided to combine all four larval instars into one single stage. This may lead to some imbalance, as there is some experimental evidence that *An. gambiae* may progress through the larval instars at different rates [104], and that there is some heterogeneity (in terms of survival probability, density dependence, and stage duration) between the instars [105,106]. Some existing models assume that the differences between sub-stages are significant, and favour incorporation of larval instars, either explicitly or implicitly, to try and represent this biological heterogeneity more realistically [11,107,108]. The decision not to separate larvae into four instars was based on a lack of experimental data on how temperature and age affect mortality of each instar stage. Further research is, therefore, needed to parameterise models that include instar-specific temperature- and age-dependent survival, although given our findings concerning the apparent greater importance of capturing age-dependent survival of adults compared to larvae, this is arguably less of a priority compared to other current data needs.

The fact that the integer value of α (the shape patameter of the gamma hazard functions) closest to the value obtained by MLE fitting differed by temperature (Supplementary Tables S1 and S2) indicates that the effect of senescence on mosquito mortality may also depend on environmental temperature. In the case where the effect of temperature on senescence is significant, the assumption of a model with a fixed number of larval or adult sub-stages may not be optimal, and a different model structure may be preferable. The models developed here were formulated as ordinary differential equations (ODEs), given that gamma-distributed processes (e.g., survival times) are mathematically convenient and computationally straightforward to express using this type of equations [72]; other waiting time distributions are considerably less convenient, or indeed possible, to model using ODEs. However, there are important limitations to modelling population dynamics using ODEs, in particular the fact that ODE-based models are typically used to represent a population-level framework, not to track individuals. This has implications for the detailed modelling of disease vectors, which may require an individual-based approach to track parity, mating, fertility (and heterogeneities therein), all of which affect vector abundance. However, while the gamma distribution is mathematically convenient to include within this framework, other waiting time distributions are considerably less convenient, or indeed possible, to model using ODEs. Due to these limitations, alternative model structures may, therefore, be more appropriate for modelling temperature-dependent development and mortality, into which the concept of age-dependence may be more readily and naturally incorporated. One such structure is the degree-day (DD) formulation, which is particularly applicable (and widely used) for modelling insects and pests as it models physiological, as opposed to chronological, age and expresses the development of poikilotherms as a function of the difference (in degrees Celsius) between the environment and the minimum temperature for development (MTD) of the insect [109–111].

In terms of parameter uncertainty, in addition to the difficulties arising from the limited availability of good quality datasets to enable robust model fitting (discussed in Section 3.1), other assumptions are

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also unavoidable given current data limitations. The mortality rates of eggs and pupae are assumed to be temperature independent on the basis that both stages are relatively short lived (compared to the larval and adult stages) and may not be significantly affected by external factors (or, if they are, the effect may be comparatively minor to strongly affect abundance prediction). To the best of our knowledge, there is currently no suitable detailed experimental data to suggest that this assumption may be erroneous, but it may be a consideration for future experimental work.

The models developed here assume that the immature and mature stages are entirely independent, but research by Christiansen-Jucht *et al*. [34,65] shows that adult parameters depend on the environmental conditions of the immature stages. While this dependency is ignored in this paper in order to focus on the role and importance of age-dependent mortality in population models of *An. gambiae*, we consider it potentially extremely important and is the subject of work in progress. In addition, since the age-dependent parameters (and some of the temperature-dependent parameters) used here are based on the same work in [34,65], it is important to note that these parameterisations are based on four temperature data points for the larval stages, and three temperature data points for the adult stages, all of which are 3 °C apart. Fitting these parameters to functional forms that reliably hold across a wider range of temperatures of interest is clearly a limitation of model parameterisation, but, as with all parameter uncertainty, is carefully considered through sensitivity analyses. In addition to the limited number of temperatures at which distributions were fitted to the experimental survival data, Christiansen-Jucht *et al*. [34] found that the Gompertz distribution generally represented the best fit. The difference between the Gompertz and gamma distributions' fits was found in [34] not to be significant for most of the temperature data points considered, and both fitted the survival data significantly better than the exponential distribution (although the Gompertz distribution yielded the best fit overall). In order to incorporate our analysis of the survival data in [34] as realistically as possible, it may be important to model Gompertz-distributed, rather than gamma-distributed, mortality.

The models developed here aim at capturing the population dynamics of *An. gambiae* in the wild, but it should be noted that the calibration of parameters is based on data obtained on laboratory mosquitoes (essentially due to the scarcity of data available on wild mosquito life history parameters). It may be that mosquitoes in the laboratory are not, in fact, truly representative of wild populations, in which case the parameters adopted here may be limited in their application to modelling *An. gambiae* population dynamics in sub-Saharan Africa, and the fit of these models to datasets used should be interpreted with caution. This highlights again the need for better entomological experimental data to inform models. We also note that the parameterisation adopted is time independent, meaning that we assumed there was no phenotypic change between mosquitoes collected in abundance datasets from different periods. However, recent research has suggested that insect vectors can evolve over decadal timescales in response to rapidly changing environmental pressures [112]. This has implications for the common model fitting assumption that different mosquito collection datasets temporally far apart are comparable; the datasets our review unearthed span a period of almost ten years, and stretch across Africa. The models constructed do not account for any spatial and/or temporal heterogeneity in model parameters, nor do they offer a way of testing for such diversity, and this common assumption represents a limitation that also potentially undermines the modelling of other mosquito-disease systems.

Since the models presented here contain a considerable number of parameters, we tried to base as many as possible on existing laboratory or field data to fix them at biologically plausible values in order

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to contain our analyses and address our research aims as succinctly as possible. It should be noted, however, that all four models are also likely to be sensitive to variations in these parameters that arise due to ongoing limitations in current entomological data quality and quantity; thus, although we have assigned these parameters to the most biologically realistic values (or environmental dependencies) based on the most up-to-date evidence and data on *An. gambiae* life history, further work is undoubtedly required to explore the implications of this additional parameter uncertainty on our findings. In this work, the decision was made to explore only the sensitivity of the models to those parameters that were not based upon experimental data, or for which the range of priors only were based on experimental data, in order to most clearly present our findings and the implications for future modelling of *An. gambiae* (and other mosquito populations for which temperature- and age-dependence has been experimentally shown to exist [34,38,53–55,61]). However, as mentioned above, there are undoubtedly considerable gaps in the current entomological data (e.g., life-history parameter dependencies derived from a wide range of environmental variations, heterogeneity between different *An. gambiae* populations due to local adaptations, relatively small sample sizes in the limited number of controlled experiments that have been undertaken, and so on), and therefore the values at which non-fitted parameters were fixed may not be as optimally representative of the mosquito's biology as we have necessarily assumed. Further sensitivity or uncertainty analyses around these parameters is therefore likely to provide additional useful insight into the extent to which models can rely on current entomological laboratory or field data to inform their predictions and where future experimental and modelling studies are ideally required.

Some of the limitations described here may lead to the effect of senescence being underestimated in our model fits. Yet, despite certain assumptions, the models that include adult-only age-dependent mortality and larval and adult age-dependent mortality still fitted the abundance data better than the other models, and we therefore expect the strength of our result concerning the importance of adult age-dependent mortality to be very conservatively reported here. Reducing the uncertainty around some of the entomological parameters, and using more detailed datasets to inform model fitting and inference, may help to highlight further the true effect and importance of including age-dependent mortality, particularly in the adult stages, in models of vector abundance. We are currently undertaking further research fitting the models presented here and other models to several abundance datasets from across sub-Saharan Africa, to determine whether age-dependent mortality varies between the geographical locations of the mosquito habitats.

Finally, we note that the modelling frameworks developed here are not limited to *An. gambiae s.s.* mosquitoes, or indeed to malaria. While these particular models are only relevant for *Anopheles gambiae s.s.* (by virtue of parameterisation based on entomological data of this species alone), dozens of other *Anopheles* species also transmit malaria [113]. There is currently no detailed experimental evidence to assess whether the life history parameters of one *Anopheles* species may be applicable to others, and this highlights the need for extremely precise experimental data on the many vectors of VBDs. However, provided detailed life-history parameters were available for other species, the framework developed here is readily applicable to other mosquito species to assess the importance and role of temperature- and age-dependent mortality on vector abundance and, ultimately, disease transmission and implications for control.

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4. Conclusions

Comparison between the models developed here suggests that including temperature- and age-dependent survival in the adult stages of models of *An. gambiae s.s.* population dynamics may provide significantly better fits to longitudinal adult female abundance data from sub-Saharan Africa. Despite the limitations and assumptions of this study, we show that models incorporating age-dependent mortality in the adult stages match temporal trends in observed mosquito data better than models without age-dependent mortality or models that include age-dependent mortality in the larval stages only. Our results indicate almost no difference in model fitting between adult-only age-dependent survival and adult and larval age-dependent survival; thus, another important conclusion of this work is that despite experimental evidence for both larval and adult age-dependent mortality, including age-dependent mortality in the adult stages only represents both the most parsimonious option for modelling and the best fit to data. This suggests that the common assumption of constant adult *An. gambiae* mortality (or even modified to incorporate temperature dependence), or, equivalently, that senescence does not play a large role in driving vector mortality, may be erroneous or, at the very least, a serious underestimation. Nonetheless, this result requires further research and confirmation, both from more detailed vector mortality data collected the field, additional experimental data from the laboratory (e.g., on other mosquito species), and further modelling studies of both the mosquito populations themselves and the diseases they transmit: in the case of malaria and other VBDs, since the inclusion of senescence in models of mosquito abundance appears to enable more accurate and reliable predictions of the population dynamics of a key malaria vector in sub-Saharan Africa, this may have important implications for VBD modelling.

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Author Contributions

Céline Christiansen-Jucht, Paul E. Parham, and María-Gloria Basáñez conceptualised the study. Céline Christiansen-Jucht and Paul E. Parham built and parameterised the model. Kamil Erguler performed the fitting. Chee Yan Shek performed the literature review. Céline Christiansen-Jucht interpreted the data and wrote the manuscript. All authors read and approved the final version of the manuscript.

Abbreviations

AIC	Akaike Information Criterion
An. gambiae s.s.	*Anopheles gambiae sensu stricto*
BIC	Bayesian Information Criterion

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CI	Credible Interval
DD	Degree-day
EMAC	ECHAM5/MESSy2 Atmospheric Chemistry
IRS	Indoor Residual Spraying
LSM	Larval Source Management
MAP	maximum *a posteriori*
MLE	Maximum Likelihood Estimation
MTD	Minimum Temperature for Development
ODE	Ordinary Differential Equation
RH	Relative Humidity
sst	sea-surface temperature
sic	sea-ice coverage
VBD	Vector-Borne Disease
°C	degrees Celsius

Conflicts of Interest

The authors declare no conflict of interest.

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Parasites & Vectors

RESEARCH Open Access

Larval and adult environmental temperatures influence the adult reproductive traits of *Anopheles gambiae* s.s.

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Abstract

Background: *Anopheles* mosquito life-history parameters and population dynamics strongly influence malaria transmission, and environmental factors, particularly temperature, strongly affect these parameters. There are currently some studies on how temperature affects *Anopheles gambiae* s.s. survival but very few exist examining other life-history traits. We investigate here the effect of temperature on population dynamics parameters.

Methods: *Anopheles gambiae* s.s. immatures were reared individually at 23 ± 1 °C, 27 ± 1 °C, 31 ± 1 °C, and 35 ± 1 °C, and adults were held at their larval temperature or at one of the other temperatures. Larvae were checked every 24 h for development to the next stage and measured for size; wing length was measured as a proxy for adult size. Females were blood fed three times, and the number of females feeding and laying eggs was counted. The numbers of eggs and percentage of eggs hatched were recorded.

Results: Increasing temperatures during the larval stages resulted in significantly smaller larvae ($p = 0.005$) and smaller adults ($p < 0.001$). Adult temperature had no effect on the time to egg laying, and the larval temperature of adults only affected the incubation period of the first egg batch. Temperature influenced the time to hatching of eggs, as well as the time to development at every stage. The number of eggs laid was highest when adults were kept at 27 °C, and lowest at 31 °C, and higher adult temperatures decreased the proportion of eggs hatching after the second and third blood meal. Higher adult temperatures significantly decreased the probability of blood feeding, but the larval temperature of adults had no influence on the probability of taking a blood meal. Differences were observed between the first, second, and third blood meal in the times to egg laying and hatching, number of eggs laid, and probabilities of feeding and laying eggs.

Conclusions: Our study shows that environmental temperature during the larval stages as well as during the adult stages affects *Anopheles* life-history parameters. Data on how temperature and other climatic factors affect vector life-history parameters are necessary to parameterise more reliably models predicting how global warming may influence malaria transmission.

Keywords: *Anopheles gambiae sensu stricto*, Environmental temperature, Larval size, Wing length, Development, Fecundity, Fertility, Blood feeding, Climate change

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Background

Climate change is expected to have a potentially important influence on the spread and transmission of vector-borne diseases, including mosquito-borne diseases such as malaria While shifts in environmental factors associated with climate change are expected to influence the global and/or local spread of malaria [1, 2], our knowledge of the precise effect of climatic factors, including temperature, on disease transmission remains limited [3, 4]. There are two main reasons for our lack of understanding.

Firstly, environmental temperature will play a complex role in the transmission of vector-borne diseases by influencing many life-history traits of both the mosquitoes and parasites that underlie transmission. It is clear, for example, that increasing mean temperatures associated with climate change may shorten the life span of the malaria vector [5, 6], and increase the developmental rate of the parasite within the mosquito [7–9], affecting transmission in opposing ways [10, 11]. It is also clear that higher temperature during larval development increases developmental rate and decreases the size of adult mosquitoes (and other cold-blooded animals) [12, 13]. Size of the mosquito can affect other parameters important for transmission such as fecundity [14, 15] (which affects mosquito population density), mating probability [16], or blood meal size [14, 17], which will be correlated with the uptake of parasites, and therefore with the probability of infection and transmission, as well as longevity and survival [18]. Mean environmental temperatures and temporal temperature fluctuations, as well as other climatic factors such as rainfall and humidity, have been shown to impact the dynamics of *Anopheles* vector populations [19, 20]. A better understanding of the effects of environmental factors on mosquito ecology, and the quantification of these effects, are therefore extremely valuable for predictions of vector abundance, distribution, evolutionary fitness, and transmission capacity [11, 21], and thereby the spread of malaria, particularly under the influence of climate change.

Secondly, the effects of temperature are usually studied during either larval development [22–24] (influencing the rate of development) or adult life (influencing life span [25]), but while it has been shown that temperatures during these two life stages can interact to influence important life-history traits such as longevity [26], no data currently exist, to the best of our knowledge, on the effect of environmental temperature to which immature stages of *Anopheles gambiae* Giles sensu stricto (s.s.) are exposed on the life and reproductive traits of the adult mosquito. A clearer and more comprehensive understanding of these interactions will allow more accurate predictions of how temperature can affect the ecology and population dynamics of the malaria vector, and thus, in turn, malaria transmission.

We present here results on the effects of environmental temperature during the larval and adult stages of *Anopheles gambiae* s.s. on several of its life-history traits; results on how temperature impacts survival have been presented elsewhere [26].

Methods

Larval maintenance and temperature regimes

Anopheles gambiae s.s. from the Kisumu colony from Western Kenya, were maintained at Imperial College London's Silwood Park campus, by blood feeding on JCK and AS's arms. After hatching, each larva was held in 3 ml of deionised water in one well of a 12-well plate at either 23 ± 1 °C, 27 ± 1 °C, 31 ± 1 °C, or 35 ± 1 °C (air temperature). An equal number of larvae (n = 640) were placed at each temperature and fed TetraMin® baby fish food according to the regimen described in [26]. As the sex of the larvae cannot be identified, we assumed a ratio of 1:1 of males to females. Larvae reared at 35 °C all died before emergence into adults. Both larvae and adults were reared in a 12:12 light/dark cycle and at 75 % (±5 %) relative humidity (RH).

Larval developmental rates and larval size

Larvae were examined for emergence to the next developmental stage (from L1 to L2, L2 to L3, L3 to L4, L4 to pupae, and pupae to adults), and photographed every 24 h to record developmental rates and larval size for seven days until all larvae pupated. The photographs were analysed and the larvae measured with the software ImageJ (ImageJ64 1.45 s, NIH, USA). Larval body length was measured from the distal tip of the head to the end of the anal segment excluding all antennae, feeding brush, and caudal hair. Larval head width (for instar determination) was measured across the widest part of the head (Additional file 1: Figure S1).

Adult maintenance and temperature regimes

In order to distinguish between the effects of larval and adult environmental temperatures, and their possible interaction, the adult mosquitoes were divided into three batches immediately upon emergence, and each batch was held in a cage at either 23 ± 1 °C, 27 ± 1 °C, or 31 ± 1 °C (as described in [26]). Adults were allowed to mate for four days, following which all males were discarded. The females were then held individually in plastic cups and fed with cotton balls soaked with a 10 % sugar solution, which were discarded 24 h before each blood meal. The females were offered blood meals on CC-J's arm at three time points: 5, 12, and 19 days after emergence in order to allow for egg laying.

Feeding, oviposition and egg hatching

The number of females feeding at each blood meal was counted, and mosquitoes that did not feed were thereafter not included in the experiment. The proportion of females imbibing blood at each blood meal and each of the adult environmental temperatures was recorded.

The bottom of each cup was filled with deionised water 24 h after each blood meal to encourage oviposition, and the adult mosquitoes were transferred to new, dry cups 48 h after laying eggs. Eggs were kept at the same environmental temperature as their mothers until hatching. The number of females that laid eggs after blood feeding, the proportion of egg-laying females among those which blood fed, the number of eggs laid by such females (a measure of fecundity), and the number of eggs that hatched among those laid (a measure of fertility) were counted. The time between blood feeding and egg laying and the time between egg laying and hatching were also recorded. Censoring occurred 35 days after the emergence of the parental generation into L1 larvae, with all mosquitoes monitored until that day (as described in [26]).

Adult female size

On day 35 after the parental generation emerged as L1 larvae, mosquitoes still alive were frozen and all adult females were measured. Both wings of each mosquito were removed, glued onto a microscope slide, photographed, and measured from the distal end of the alula to the tip with the software ImageJ (Additional file 1: Figure S2). As mosquitoes were not followed until their natural death, longer-lasting effects of the temperature of the maternal environment may have been lost. Data on the effect of temperature during the immature and mature stages on mosquito survival have been presented elsewhere [26] and will not be considered further here.

Statistical methods

Larval developmental rates and larval size

A non-parametric (Kaplan-Meier) survival analysis was performed on the time for each mosquito stage to develop to the next larval, pupal, or adult stage, to allow for a comparison of how different temperature regimens during the immature stages affected the time to development from L1 to L2, L2 to L3, L3 to L4, L4 to pupae (P), and pupae to adults (A). The number of cumulative days to development for the L1–L2, L2–L3, L3–L4, L4–P, and P–A transitions was also calculated. Larval body size was measured from the distal tip of the head, excluding antennae, feeding brush, and caudal hair, to the end of the anal segment [27]. For each larval instar, the arithmetic mean of larval sizes was taken. Tukey's test was used to compare mean instar-specific larval sizes between pairs of larval temperature regimens. For each of the seven days the larvae were followed to pupation, the arithmetic mean of larval sizes across all larval stages (from L1 to L4) were compared across all four larval temperatures using the F statistic.

Blood feeding and oviposition

The effect of larval and adult environmental temperature on the probability of female mosquitoes taking blood meals (measured as the proportion of females imbibing blood from the total that were offered a blood meal at each adult temperature), and on the probability of oviposition after each blood meal (measured as the proportion of females ovipositiong among those which had), was analysed by logistic regression and compared using the log-likelihood ratio test.

The mean number of eggs laid by female mosquitoes after each blood meal was analysed by Tukey's test to investigate the influence of different larval and adult temperatures, and temperature combinations, on the number of eggs produced by adult females at each temperature regimen. Eggs laid by females remained at the same environmental temperature as the mothers, and the mean proportion of eggs hatching at each environmental temperature (23 °C, 27 °C, and 31 °C) were compared using a Mann-Whitney test. The mean number of days between blood meal intake and egg laying, and between oviposition and hatching, were compared using the Mantel-Cox test for pair-wise comparisons between temperatures and by log-rank test for the comparison over all temperatures.

Adult female size

Adult wing length was used as a proxy for body size [28–32]. The length of both wings was measured and their arithmetic mean taken as the 'size' of each mosquito. A one-way analysis of variance (ANOVA) was used to determine the effect of larval temperature on adult wing length.

All analyses were carried out using R, Version 2.10.1 (R Foundation for Statistical Computing, 2009) [33]. Since the test statistics and their values are provided in the tables of the main text or the Additional file 1, only the corresponding *p*-values are given in the description of the results below.

Results

The effect of larval environmental temperature on larval developmental rates and size

When males and females are combined, higher temperature from 23 °C to 31 °C slowed the development from hatching to emerging (Fig. 1). No mosquitoes emerged at 35 °C. The effect of temperature differed among the larval stages. Increasing temperature increased the developmental rates of first instar larvae and of pupae, but decreased the developmental rates of fourth instar larvae. Considering only the

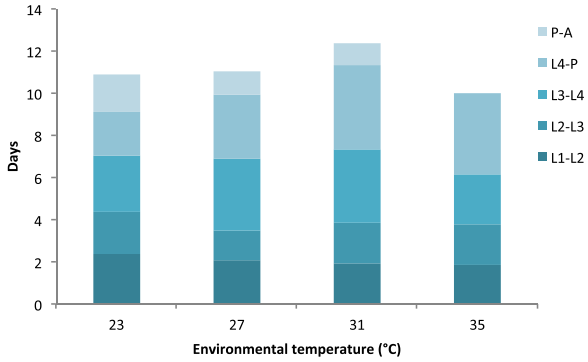


Fig. 1 Cumulative number of days to development of each life stage in *Anopheles gambiae* s.s.. Larval stages are L1, L2, L3 and L4; pupae are denoted by P and adults by A. Larvae kept at 35 ± 1 °C died, so that imagoes did not emerge from their pupal cases at this temperature

female mosquitoes, the age at emergence increased with increasing temperature from 10.92 days at 23 °C to 12.35 days at 31 °C. The results of the Kaplan-Meier analyses are presented in Fig. 2.

Figure 3 shows the daily increase in larval size (averaging across all larval stages, from L1 to L4) at all four environmental temperatures for seven days, by which time all larvae pupated. The overall effect of temperature on larval size was statistically significant ($p = 0.005$). Higher temperatures resulted, overall, in smaller larvae on days 1 ($p < 0.001$), 2 ($p < 0.001$), 4 ($p < 0.001$), 5 ($p = 0.006$), 6 ($p < 0.001$) and 7 ($p < 0.001$), with an increase observed on day 3 ($p < 0.001$). The values of the pair-wise comparisons between mean larval sizes for larvae reared at every

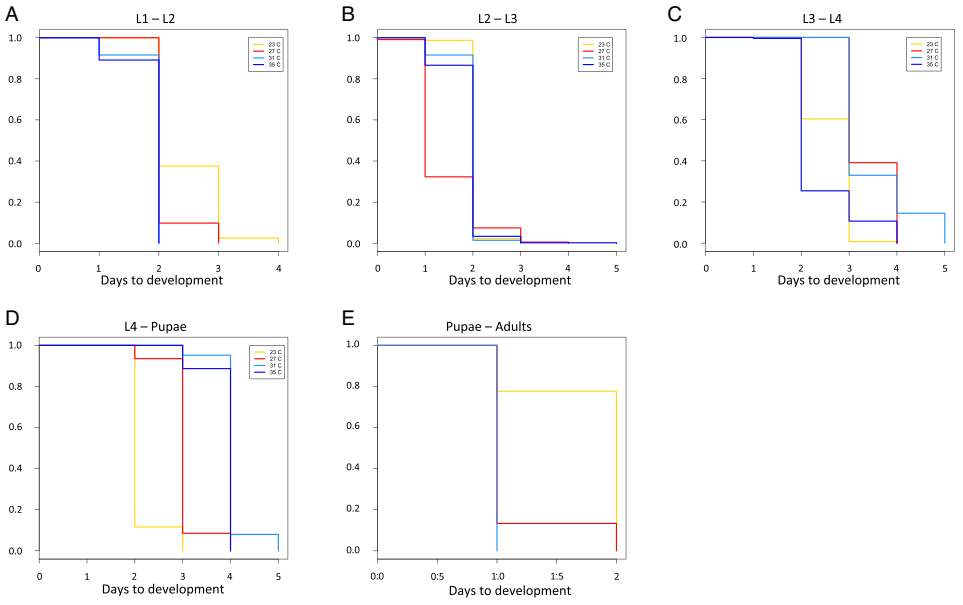


Fig. 2 Kaplan-Meier plots of the stage-specific survival analysis of development times. **a** Development time from L1 to L2 larvae. **b** Development time from L2 to L3 larvae. **c** Development time from L3 to L4 larvae. **d** Development time from L4 larvae to pupae. **e** Development time from pupae to adults. Coloured lines represent the environmental temperatures investigated as described in Fig. 1

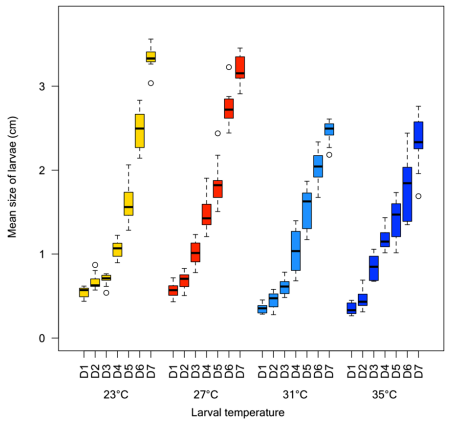


Fig. 3 Mean larval size by day as a function of environmental temperature. Size of larvae (in cm) by day post-hatching (D1 is 24 h post-hatching, D2 48 h post-hatching, etc.) and by temperature (23 ± 1 °C: yellow; 27 ± 1 °C: red; 31 ± 1 °C: light blue; 35 ± 1 °C: dark blue). The bottom and top of the boxes describe the first and third quartiles respectively, and the bold line inside the boxes represents the median. The whiskers show the data minima and maxima, and the small circles represent outliers

temperature regimen, as well as test statistics for the overall comparisons across temperatures are given in Additional file 1: Table S1.

The effect of larval and adult environmental temperature on blood feeding rates

The temperature at which adult mosquitoes were reared as larvae showed no statistically significant effect on the probability of females taking a second blood meal, although females reared at 31 °C were significantly less likely to take a third blood meal than females reared at 23 °C ($p < 0.001$) and females reared at 27 °C ($p = 0.002$) (Additional file 1: Table S2).

All females fed the first time they were offered a blood meal. However, the environmental temperature at which adults were kept had a significant effect on the probability of the mosquitoes feeding for a second and third time (Table 1). For the second blood meal, increasing the temperature from 23 °C to 27 °C decreased the probability of feeding from 0.86 to 0.76 ($p = 0.049$), as did an increase from 23 °C (0.86 probability of blood feeding) to 31 °C (0.64 probability of blood feeding) ($p < 0.001$). An increase from 27 °C to 31 °C did not significantly reduce feeding rates (0.76 at 27 °C vs. 0.64 at 31 °C ($p = 0.065$). For the third blood meal, increases from 23 °C to 27 °C, 27 °C to 31 °C, and 23 °C to 31° all significantly decreased the probability of females feeding on blood (0.48 vs. 0.22, $p < 0.001$; 0.22 vs.0.04, $p = 0.002$; and 0.48 vs.0.04, $p < 0.001$, respectively).

The effect of larval and adult environmental temperature on egg laying, number of eggs laid, and egg hatching

Rearing larvae at higher temperatures significantly decreased the probability of egg laying for adult females kept at 23 °C and 27 °C, but not for adults kept at 31 °C, after the first two blood meals (Table 2; all females laid eggs after the third blood meal). The temperature of the adult environment had no effect on the probability of female mosquitoes laying eggs after their first or third blood meal; however, after the second blood meal, an increase from 27 °C to 31 °C and from 23 °C to 31 °C resulted in a significantly lower probability of laying eggs (0.65 vs. 0.46, $p = 0.022$, and 0.72 vs. 0.46, $p = 0.002$, respectively).

The temperature at which mothers were reared as larvae significantly influenced the number of eggs laid at 23 °C and 31 °C after the first blood meal, but had no effect at 27 °C – a similar result to the effect of temperature on the time to egg laying (see below). After the second blood meal, females reared at 27 °C and 31 °C laid significantly fewer eggs than those reared at 23 °C among adults kept at 23 °C. Females reared at 31 °C also laid fewer eggs than females reared at 27 °C among adults kept at 27 °C. Among adult mosquitoes at 23 °C, females reared at 27 °C also laid significantly fewer eggs than females reared at 23 °C. The temperature at which females were reared as larvae had no impact on the number of eggs laid after the third blood meal (Table 3).

Figure 4 shows the effect of the environmental maintenance temperature of adult females on the number of

Table 1 Two-group comparisons and overall trend of the effect of increasing adult environmental temperature on the odds of blood feeding

	Test statistic	27 °C ± 1 (with respect to 23 °C)	31 °C ± 1 (with respect to 23 °C)	31 °C ± 1 (with respect to 27 °C)
2nd blood meal	Log odds of feeding	−0.706	−1.272	−0.566
	log-likelihood ratio test	−1.972	−3.509	−1.844
	p-value	0.049	<0.001	0.065
3rd blood meal	Log odds of feeding	−1.188	−2.959	−1.771
	log-likelihood ratio test	−4.004	−5.397	−3.177
	p-value	<0.001	<0.001	0.0015

Table 2 Two-group comparisons and overall trend of the effect of increasing larval environmental temperature on the odds of laying eggs by *Anopheles gambiae* s.s. females kept at all adult temperatures for the first two blood meals[a]

Adult temperature (°C)			Larval temperature (°C)		
			27 ± 1 (with respect to 23 °C)	31 ± 1 (with respect to 23 °C)	31 ± 1 (with respect to 27 °C)
1st blood meal		Log odds of laying eggs	−0.783[b]	−1.658	−0.876
	23 ± 1	log-likelihood ratio test	−1.187	−2.455	−1.515
		p-value	0.235	0.014	0.130
	27 ± 1	Log odds of laying eggs	−2.565	−4.069	−1.504
		log-likelihood ratio test	−2.383	−3.828	−3.086
		p-value	0.017	<0.001	0.002
	31 ± 1	Log odds of laying eggs	0.767[c]	−0.028	−0.795
		log-likelihood ratio test	1.386	−0.048	−1.392
		p-value	0.166	0.962	0.164
2nd blood meal		Log odds of laying eggs	−1.952	−3.312	−1.36
	23 ± 1	log-likelihood ratio test	−2.398	−3.805	−2.276
		p-value	0.016	<0.001	0.023
	27 ± 1	Log odds of laying eggs	−1.161	−2.496	−1.335
		log-likelihood ratio test	−1.613	−3.486	−2.534
		p-value	0.107	<0.001	0.011
	31 ± 1	Log odds of laying eggs	1.121	0.359	−0.762
		log-likelihood ratio test	1.557	0.465	−1.218
		p-value	0.120	0.642	0.223

[a]All females laid eggs after the third blood meal. [b]A negative value of the log odds represents a decrease in the proportion of females laying eggs with respect to the reference temperature, whilst a positive value[c] represents an increase in egg laying rate

eggs laid after each blood meal. After the first blood meal, females held at 23 °C and 27 °C laid 43 ± 2 eggs (mean ± SE) and 50 ± 2 eggs ($p = 0.036$) respectively, while females at 31 °C laid significantly fewer eggs than females at both of the other regimens (25 ± 2 eggs; $p < 0.001$ in both comparisons). After the second blood meal, females at 23 °C and 27 °C laid 47 ± 2 eggs and 47 ± 4 eggs ($p = 0.953$) respectively, while females at 31 °C laid 18 ± 3 eggs ($p < 0.001$). After the third blood meal, females at 23 °C and 27 °C laid 47 ± 2 eggs and 51 ± 6 eggs ($p = 0.365$), whereas females at 31 °C laid 10 ± 7 eggs ($p < 0.001$ with respect to 23 °C and $p = 0.007$ with respect to 27 °C).

Larval rearing temperatures of mothers had no effect on the mean proportion of laid eggs that hatched into larvae for the first and third blood meals. After the second blood meal, the mean proportion of eggs laid by females reared at 27 °C and 31 °C and that hatched, was significantly higher in comparison with the hatching rate of those eggs laid by females reared at 23 °C (Additional file 1: Table S2). Because the larval temperature at which the mothers had been reared had no impact otherwise on the mean proportion of eggs that hatched, this is likely to be a chance event. The temperature at which the females spent their adult life did impact the proportion of offspring hatching after the second and third blood meal (Table 4).

Time to egg laying and time to egg hatching

Table 5 shows the effect of the environmental temperature at which larvae were reared on the time between blood feeding and egg laying of the resulting female mosquitoes, for each blood meal. The temperature at which females were reared as larvae influenced the time to egg laying following the first blood meal only, when an increase in

Appendix C

Table 3 Two-group comparisons and overall trend of the effect of increasing larval environmental temperature on the mean number of eggs laid by adult *Anopheles gambiae* s.s. females (reared from these larvae) kept at all adult temperatures. No mosquitoes held at 31 °C survived long enough to blood feed for a third time

Adult temperature (°C)			Larval temperature (°C)			
			23 ± 1	27 ± 1 (with respect to 23 °C)	31 ± 1 (with respect to 23 °C)	31 ± 1 (with respect to 27 °C)
1st blood meal		Mean n° of eggs (±SD)	53.11 (2.66)	41.39 (2.98)	22.31 (5.79)	22.31 (5.79)
	23 ± 1	Test statistic (*p*-value)		2.941 ($p < 0.05$)	5.501 ($p < 0.01$)	3.212 ($p < 0.05$)
	27 ± 1	Mean n° of eggs (±SD)	48.67 (2.86)	52.77 (4.09)	48.35 (5.14)	
		Test statistic (*p*-value)		−0.846 (0.4)	0.057 (0.95)	0.662 (0.51)
	31 ± 1	Mean n° of eggs (±SD)	39.00 (3.9)	23.17 (2.77)	14.29 (3.38)	
		Test statistic (*p*-value)		3.337 ($p < 0.01$)	4.719 ($p < 0.001$)	1.899 (0.064)
2nd blood meal		Mean n° of eggs (±SD)	57.53 (3.23)	38.6 (3.55)	30.71 (2.21)	
	23 ± 1	Test statistic (*p*-value)		3.929 ($p < 0.001$)	3.803 ($p < 0.001$)	1.146 (0.261)
	27 ± 1	Mean n° of eggs (±SD)	46.57 (5.57)	57.96 (4.60)	28.17 (8.05)	28.17 (8.05)
		Test statistic (*p*-value)		−1.584 (0.12)	1.907 (0.065)	3.455 ($p < 0.01$)
	31 ± 1	Mean n° of eggs (±SD)	29.75 (4.23)	13.73 (4.19)	22.00 (5.71)	
		Test statistic (*p*-value)		1.88 (0.077)	0.932 (0.376)	−1.136 (0.269)
3rd blood meal		Mean n° of eggs (±SD)	50.54 (2.47)	44.6 (2.58)	36.00 (3.96)	
	23 ± 1	Test statistic (*p*-value)		1.657 (0.1)	2.535 ($p < 0.05$)	1.548 (0.135)
	27 ± 1	Mean n° of eggs (±SD)	54.18 (7.08)	47.20 (6.60)	50.75 (28.21)	
		Test statistic (*p*-value)		0.76 (0.457)	0.173 (0.866)	−0.186 (0.855)

larval environmental temperature from 23 °C to 27 °C and from 23 °C to 31 °C resulted in a significantly longer gonotrophic development (from 4.03 to 4.15 days ($p = 0.03$) and from 4.03 to 4.29 days ($p < 0.001$), respectively). Larval temperature did not influence the duration of time for females to lay eggs after the second (average duration of 4.0 days) or third blood meal (average duration of 3.45 days). At 23 °C, the average duration of time for females to lay eggs was significantly shorter after the third blood meal (3.57 days) than after the first (4.03 days, $p = 0.001$) or second blood meal (4.0 days, $p = 0.02$). At 27 °C, the time to oviposition was also significantly shorter after the third blood meal (3.31 days) than after the first (4.15 days, $p < 0.001$) or second blood meal (4.03 days, $p < 0.001$). At 31 °C, the time to egg laying was significantly shorter after the second blood meal (4.0 days) than after the first blood meal (4.29 days, $p = 0.004$), and after the third blood meal (3.44 days) than after the first blood meal (4.29 days, $p < 0.001$), but there was no significant difference between the third (3.44 days) and second blood meal (4.0 days, $p = 0.057$).

Rearing adults at warmer temperatures had no effect on the time to oviposition for the first, second, or third batch of eggs (Additional file 1: Table S3). However, at all adult environmental temperatures considered, the duration between blood feeding and oviposition became significantly shorter after each blood meal (23 °C: $p < 0.001$; 27 °C: $p < 0.001$; 31 °C: $p = 0.027$; Additional file 1: Table S4).

The effect of the environmental temperatures on time to eggs hatching is shown in Table 6. Keeping the eggs at warmer temperatures significantly quickened to hatching of the first and second batch of eggs ($p < 0.001$ in both

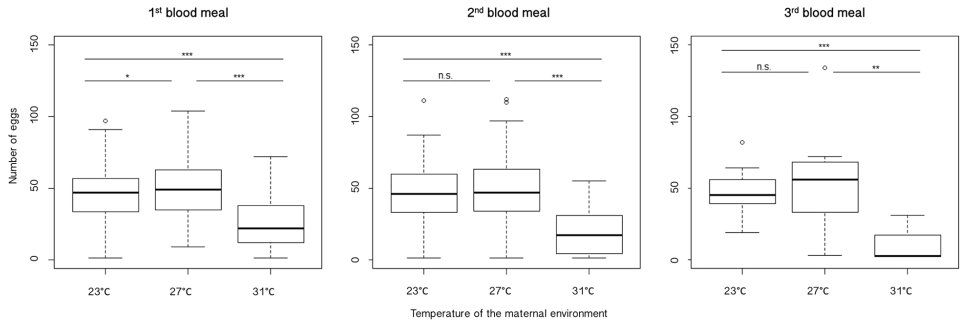


Fig. 4 Number of eggs laid at each adult temperature after each blood meal. The bottom and top of the boxes describe the first and third quartiles respectively, and the bold line inside the boxes represents the median. The whiskers show the data minima and maxima, and the small circles represent outliers. (n.s.: not significant; *: $p < 0.05$; **: $p < 0.01$; ***: $p < 0.001$)

cases), but a temperature of 31 °C had no effect on the time to hatching of the eggs produced by the third blood meal ($p = 0.69$).

For eggs produced after the first blood meal, a 4 °C increase in the environmental temperature of the eggs from 23 °C to 27 °C significantly shortened the time to hatching from an average of 1.72 days to 1.07 days ($p < 0.001$), but a 4 °C increase from 27 °C to 31 °C increased the time to hatching from 1.07 to 1.75 days ($p < 0.001$). The difference in hatching time between the coldest (23 °C) and the warmest environmental temperature (31 °C) was not significant ($p = 0.82$).

After the second blood meal, a 4 °C increase from 23 °C to 27 °C significantly shortened the time to hatching, from 2.15 days to 2.11 days ($p < 0.001$), whereas a 4 °C increase from 27 °C to 31 °C had no effect on the time to hatching (2.11 days and 2 days respectively, $p = 0.91$). The 8 °C increase from 23 °C to 31 °C, therefore, also significantly shortened the time to hatching ($p < 0.001$).

The temperature at which adult females were reared as larvae was found to have no effect on the time to hatching of their eggs (Additional file 1: Table S5).

The effect of larval environmental temperature on adult mosquito size

The mean wing lengths of adult mosquitoes reared at 23 °C, 27 °C, or 31 °C as larvae are shown in Fig. 5. There is a significant trend for the size of adult female mosquitoes to decrease with increasing larval environmental temperature (overall $p < 0.001$). Every increase in larval temperature brought about a significant decrease in size (27 °C vs. 23 °C; 31 °C vs. 23 °C; and 31 °C vs. 27 °C, all p-values <0.001; Additional file 1: Table S6). Temperature of the adult environment was found not to have any effect on the wing length of adult mosquitoes (F-statistic = 0.17, $p = 0.844$).

Discussion

The temperature experienced by *Anopheles gambiae* s.s. during all of its life stages, from eggs through to adults, is shown here to affect several key life-history parameters of considerable importance for a better understanding of the impact of climatic variables on mosquito population dynamics, namely, larval and adult size, development rate of each life stage, propensity to blood feed, number of eggs

Table 4 Effect of adult environmental temperature on the proportion of laid eggs that hatched after each blood meal taken by *Anopheles gambiae* s.s.

		23 ± 1 °C	27 ± 1 °C (with respect to 23 °C)	31 ± 1 °C (with respect to 23 °C)	31 ± 1 °C (with respect to 27 °C)
1st blood meal	Proportion	0.960	0.964	0.961	
	Test statistic (*p*-value)		−1 (0.757)	−0.17 (0.690)	0.58 (0.825)
2nd blood meal	Proportion	0.890	0.903	0.742	
	Test statistic (*p*-value)		−1.63 (0.784)	8.73 (0.007)	9.78 (0.019)
3rd blood meal	Proportion	0.810	0.795	0.632	
	Test statistic (*p*-value)		1.07 (0.315)	2.76 (0.010)	2.44 (0.043)

Table 5 Two-group comparisons and overall trend of the effect of increasing larval environmental temperature on the time to egg laying by adult *Anopheles gambiae* s.s. females reared from the larvae

		23 °C ± 1	27 °C ± 1 (with respect to 23 °C)	31 °C ± 1 (with respect to 23 °C)	31 °C ± 1 (with respect to 27 °C)	Overall effect of larval temperature on time to egg laying	
1st blood meal	Days (±SD)	4.03 (±0.18)	4.15 (±0.36)	4.29 (±0.46)			
	Mantel-Cox test statistic		4.51	11.08	2.20	Log-rank test statistic	11.06
	p-value		0.03	<0.001	0.14	*p*-value	<0.001
2nd blood meal	Days (±SD)	4.00	4.03 (±0.18)	4.00 (±0.0)			
	Mantel-Cox test statistic		0.48	0	0.50	Log-rank test statistic	0.05
	p-value		0.49	0.99	0.48	*p*-value	0.83
3rd blood meal	Days (±SD)	3.57 (±0.5)	3.31 (±0.53)	3.44 (±0.53)			
	Mantel-Cox test statistic		2.02	0.22	0.19	Log-rank test statistic	2.03
	p-value		0.16	0.64	0.67	*p*-value	0.36

laid (fecundity) and proportion of these that hatch (fertility). Our results complement, through more detailed experiments, scarce studies in this area and, importantly, examine the question of how the environmental temperature of the immature, aquatic stages, may influence life-history traits of the adult, aerial stages.

Our results confirm those of previous studies [13, 34, 35] reporting that the temperature of the larval environment significantly influences both larval and adult size, with higher temperatures resulting in smaller larvae and adults. This has important implications for predictions of malaria spread under warmer climate scenarios, as the size of *Anopheles gambiae* s.s. has been shown to be an important determinant of its vectorial capacity for malaria transmission [36]. However, to our knowledge, there are currently very few data on the influence of environmental temperature during both the juvenile and adult stages on the life-history parameters of *Anopheles gambiae* s.s..

The average time spent in each immature stage was influenced by environmental temperature, but the direction of the effect was different for the earlier (shortening the time until the L3–L4 stage transition) and later stages (lengthening the time from L3–L4 larvae to imagoes), with the net result that higher temperatures resulted in a longer development time from egg to imago. Bayoh and Lindsay [23] indicated that increasing temperature results in faster development rates which peak, in their case, at 28 °C. Under our experimental conditions, we observed the fastest development rate at 31 °C. Bayoh and Lindsay [23] also remarked that no imagoes emerged at temperatures above 34 °C, which agrees with our findings that

Table 6 Two-group comparisons and overall trend of the effect of increasing the environmental temperature of eggs on their time to hatching

		23 ± 1 °C	27 ± 1 °C (with respect to 23 °C)	31 ± 1 °C (with respect to 23 °C)	31 ± 1 °C (with respect to 27 °C)	Overall effect of larval temperature on time to egg hatching	
1st blood meal	Days (±SD)	1.72 (±0.48)	1.07 (±0.26)	1.75 (±0.81)			
	Mantel-Cox test statistic		61.88	0.05	41.05	Log-rank test statistic	78.49
	p-value		<0.001	0.82	<0.001	*p*-value	<0.001
2nd blood meal	Days (±SD)	2.43 (±0.65)	1.83 (±0.62)	1.92 (±0.49)			
	Mantel-Cox test statistic		17.71	12.81	0.01	Log-rank test statistic	21.37
	p-value		<0.001	<0.001	0.91	*p*-value	<0.001
3rd blood meal	Days (±SD)	2.89 (±0.83)	2.45 (±1.1)	3 (±0.0)			
	Mantel-Cox test statistic		0.75	0.03	0.02	Log-rank test statistic	0.75
	p-value		0.39	0.87	0.9	*p*-value	0.69

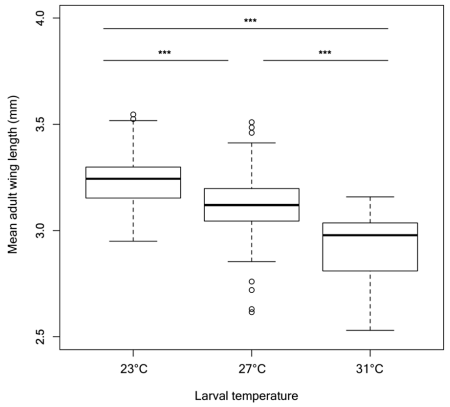


Fig. 5 Adult wing length (mm) as a function of larval environmental temperature. The bottom and top of the boxes describe the first and third quartiles respectively, and the bold line inside the boxes represents the median. The whiskers show the data minima and maxima, and the small circles represent outliers. (***: $p < 0.001$)

larvae and pupae reared at 35 °C died before they could develop into adults.

Our data also suggest there may be an optimal temperature for the gonotrophic development of *Anopheles gambiae* s.s. egg batches. Adults which emerged from immatures kept at 27 °C, and which were themselves kept at 27 °C, laid the most eggs, whereas those reared and kept at higher (and lower) temperatures laid significantly fewer eggs. Interestingly, the temperature at which mothers had been reared as larvae influenced the numbers of eggs laid after the first two blood meals, suggesting that this was not simply an effect of adult size. Higher adult temperatures resulted in a lower probability of adult females taking blood meals. When temperature affected fecundity, higher temperatures also decreased the probability of laying eggs.

Overall, increasing adult temperature did not influence the duration of the time between blood feeding and egg laying, but the duration of the gonotrophic development was significantly shortened with each additional blood meal for each temperature. The environmental temperature at which the eggs were kept did not influence the time to hatching in a discernible trend, in keeping with [37] and suggesting that while temperature may affect the time to egg hatching, the effect is minimal within an optimal range for egg development (24-30 °C), which includes the temperatures considered here.

Previous studies have examined the relationship between temperature and the development rate of other *Anopheles* species [11, 13, 22, 35, 38], as well as other mosquito vectors [39]. Research elsewhere has also looked at the effect of temperature on the gonotrophic cycle, fecundity, hatching rate, and sex ratio [35, 40], as well as the relationship between larval food quantity and several life-history parameters [41]. Our results agree with those of [37], showing that temperature can influence both the time to hatching and hatching counts.

The experimental design presented here did not account for the diurnal and nocturnal fluctuations in temperature and humidity that would affect mosquito development and survival in the field. The effects of other climatic and environmental factors on *An. gambiae* reproductive and life-history parameters, as well as the influence of local air temperature fluctuations on the water temperature of mosquito breeding sites [42, 43], require further research. In addition, *Anopheles gambiae* s.s. is only one of the seven major vectors of human malaria in Africa [44] and available data on the sensitivity of the other species to climatic variables such as temperature, as well as population-related factors, are even more scarce than data on *Anopheles gambiae* s.s.. Climate change and global warming are expected to influence the reproductive and life-history parameters [22] of different mosquito species in different ways [45]. More comprehensive data specific to each malaria vector species are required to define the dependency of mosquitoes' population dynamics on climate-related variables, which, combined with regional climate data predictions, will allow sound and dependable forecasting of disease vector population dynamics and transmission patterns.

Conclusions

There is currently considerable uncertainty about how climate change will affect temperatures and temperature fluctuations, and how this will, in turn, impact the population dynamics of disease vectors. An understanding of how short- and long-term climate change-induced temperature variations will affect the life-history parameters of disease vectors and influence their population dynamics and geographical spread is necessary for more robust and dependable forecasts of disease transmission. To this end, the results of the experimental work described here have been recently used to parameterise and fit mathematical models of *Anopheles gambiae* s.s. population dynamics to mosquito abundance data. This is a first step towards the development of more detailed and robust frameworks to better understand the effects of a warmer world on the distribution and density of mosquito vectors of disease.

Additional file

Additional file 1: Description of mosquito larvae and adult wing size measurements and additional data tables. (DOC 329 kb)

Abbreviations

ANOVA: Analysis of variance; RH: Relative humidity.

Appendix C

Competing interests
The authors declare they have no competing interests.

Authors' contributions
CDC-J, PEP, AS, M-GB, and JCK conceptualized the experimental design and study. CDC-J carried out the experiments in JCK's laboratory, and analysed the data. CDC-J wrote the first draft of the manuscript. PEP, M-GB, and JCK critically reviewed the manuscript and contributed with intellectual input. All authors read and approved the final version of the manuscript.

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Appendix C

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