Investigating The Impact Of Temperature Variations On African Trypanosome Transmissibility Within The Vector Tsetse Flies

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INVESTIGATING THE IMPACT OF TEMPERATURE VARIATIONS ON AFRICAN TRYPANOSOME TRANSMISSIBILITY WITHIN THE VECTOR TSETSE FLIES

Sophie Genigeorgis

A Thesis Submitted in Partial Fulfillment of the Requirements for the Degree of Master of Public Health 2024

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Abstract:
African trypanosomiasis, a neglected vector-borne parasitic disease transmitted by tsetse flies, poses a significant threat to human and animal health in Sub-Saharan Africa. As the effects of climate change alter weather patterns, these shifts in climate are predicted to impact the disease distribution and transmission dynamics through alterations in tsetse vector life history traits and infection prevalence.

This study investigates the potential effects of increasing temperatures on tsetse fly life history traits and their subsequent influence on African trypanosomiasis transmission. Specifically, the research examines how temperature variations impact fly survival, female fecundity, generation time, and vector competence. By analyzing these factors, the study aims to provide valuable insights into the potential consequences of climate change on African trypanosomiasis transmission dynamics and vector population demographics.

To examine these varying parameters, tsetse flies (Glossina morsitans morsitans) were reared at two different temperatures (27°C, and 28.5°C) and findings were compared to those at the ambient temperature 25°C to simulate potential temperature change scenarios. Fly survival, pupa deposition, eclosion rate, and infection prevalence with Trypanosoma brucei brucei parasites were monitored over time. Statistical analyses were performed to assess the significance of the observed differences between different temperature groups. This study found that under varying temperature conditions, tsetse fly life history traits were significantly impacted as flies reared at higher temperatures exhibited shorter development times during larval as well as accelerated development and maturation. Temperature-treated flies displayed reduced lifespans, leading to reduced output at the population level. Temperature was shown to increase the infection index of 27°C flies, but not the 28.5°C flies, suggesting a potential tipping point in parasite development and load within the fly under warming conditions that have implications for transmission rates in the future.

This research provides valuable insights into the potential consequences of climate change for African trypanosomiasis transmission patterns. While temperature increases might lead to faster development in the immature stages of tsetse flies, they may also decrease overall adult fly lifespan, creating a complex scenario for transmission dynamics that requires further research. These findings can assist in predicting future changes in transmission dynamics within Africa, informing strategies for controlling African trypanosomiasis in a changing climate.
Acknowledgments:

I am deeply indebted to my advisor and principal investigator, Dr. Serap Aksoy, for her exceptional guidance, expertise, and for providing the opportunity to conduct this research in her lab. Her mentorship has been indispensable in shaping my scientific understanding and research skills. I am immensely thankful to everyone at the Aksoy lab for their training, support, and active involvement in this experiment. Their collective efforts have contributed significantly to the realization of this project.

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I would like to acknowledge the Hortman Scholarship committee for their generous support, which enabled me to pursue my studies at the Yale School of Public Health. Special thanks to the Yale School of Public Health for providing the resources and facilities crucial to the execution of this research.

Lastly, I express my deepest appreciation to my mother and grandfather. Their unwavering sacrifices and love taught me no dream is too big.
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Temp_27 had the highest infection index. Flies reared at Temp_28.5°C experienced the smallest change in infection index from day 21 to day 28 (1% increase) and flies reared at Temp_25°C experienced a drop of 5% in infection index from day 21 to day 28.

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**Introduction:**

African trypanosomiasis, also known as Sleeping Sickness, is a neglected vector-borne, parasitic disease in Sub-Saharan Africa. The human disease, Human African trypanosomiasis (HAT), impacts 70 million people in sub-Saharan Africa and is transmitted solely by tsetse flies (Diptera:Glossina spp.) (Simarro, P.P et al, 2012). In addition to HAT, Animal African Trypanosomiasis (AAT) results in severe economic losses and restricts nutrient availability for animals and humans continent-wide (Aksoy et al, 2017). Since the early 20th century, the world has witnessed three major HAT epidemics (Steverding et al, 2008). The most recent Hat epidemic that started in the 1980s resulted in over 500,000 deaths from HAT until the epidemic was under control in early 2000 (Steverding et al, 2008). Today, AAT is widely rampant on the continent and still serves as a major public health problem (Aksoy et al, 2017).

Belonging to the genus Trypanosoma, these single-celled protozoans possess a complex, intricate life cycle involving both mammalian hosts and the vector tsetse flies (Glossina spp) (Aksoy et al, 2017). The two main species responsible for Human African Trypanosomiasis (HAT), *T. b. gambiae* and *T. b. rhodesiense*, exhibit distinct geographical distributions and clinical characteristics, with both leading to devastating neurological damage and death if left untreated (Ponte-Sucre et al, 2016). Across East and Southern Africa, *T. b. Rhodesiense* is responsible for the acute Rhodesiense form of sleeping sickness (Ponte-Sucre et al, 2016). In contrast, Central and West Africa see the chronic Gambiense form caused by *T. b. gambiae*, accounting for roughly 95% of reported HAT cases on the continent (Aksoy et al, 2017).

Due to a process of antigenic variation the parasite displays in its mammalian host, no mammalian vaccines exist (Aksoy et al, 2017). Effective and affordable oral drugs and diagnostic kits that can be used in the field are also lacking (Bonnet et al, 2015). Control of HAT caused by *T. b. Gambiae* in west-Africa has relied heavily on active case detection and treatment programmes (Franco et al, 2014). Control of HAT caused by *T. b. rhodesiense* in east-Africa is more complex as it involves animal reservoirs and hence requires vector control applications (Aksoy et al, 2017). The majority of vector control depends on the use of traps and targets and insecticide-based applications and there is desire to expand the vector control tool-box for better and effective disease control (Aksoy et al, 2017).

Infection of *T. b. brucei* occurs through tsetse flies following acquisition of blood meals from infected humans or animals and subsequently transmitting them to new mammalian hosts. Following an infective blood meal containing the bloodstream form of *Trypanosoma brucei spp.*, trypanosomes differentiate quickly to insect stage procyclic cells, and initially colonize the midgut of susceptible flies, rapidly multiplying through binary fission for 6-10 days (Geirger et al, 2014). The procyclic trypomastigotes then differentiate into epimastigotes that colonize the anterior midgut. For yet unknown reasons, in some, but not all of the midgut infected flies, the parasites progress and invade the salivary glands of the fly by 18-25 days and undergo
differentiation into early and mature stages of mammalian infective metacyclic trypomastigotes, which are ready for transmission to the next mammal during a blood meal in fly saliva. This entire life cycle, from the initial blood meal acquisition to metacyclic trypomastigotes, lasts around 30 days, constituting the extrinsic incubation period (EIP) and crucially determining transmission risk (World Health Organization).

It is important to note that not all infected blood meals yield an established infection in tsetse flies. Typically, less than 50% of flies that take a trypanosome-infected blood meal under optimal laboratory conditions become infected (Geiger et al, 2014). This is due to the natural refractoriness present in tsetse flies that serves as a form of resistance to trypanosome infections (Geiger et al, 2014). In addition, it has been noted that flies are most susceptible to infection if the parasite is acquired in the first blood meal as a newly emerged teneral fly and are rendered more resistant as adults (Geirger et al, 2015). Also of interest, not all midgut infections give rise to mature salivary gland infections. Typically under laboratory infection conditions, only about 50% of midgut infected flies give rise to salivary gland infections for mammalian transmissibility (Bateta et al, 2017).

**Transmission of Human African Trypanosomiasis:**
Transmission of HAT relies on a delicate interplay between humans (host), tsetse flies (vector), and reservoirs of parasites such as domestic animals or wildlife (Venturelli et al, 2022). Interrupting transmission involves a combination of reducing parasite reservoirs, vector control, and minimizing human-tsetse interactions (Venturelli et al, 2022).

The geographical range of tsetse flies is determined/regulated by several factors such as climate, vegetation, and host availability. Transmission probability rises with increasing tsetse fly density, wider distribution of fly populations, and a higher prevalence of infected flies (Adungo et al, 2020). Due to lack of mammalian vaccines, disease control efforts rest upon active/passive case detection, treatment of human cases, and combined vector control (Aksoy et al, 2017).

Many models demonstrate that integrating annual vector control deployed in communal areas in combination with annual or biennial active case detection and treatment can dramatically reduce the burden and prevalence of *Gambiense* HAT (Pandey et al, 2015). These models have been reinforced through studies such as Courtin et al, (2015) that demonstrate incidence is 10 times higher in areas without vector control. For low-endemicity settings, vector control is a cost-effective alternative to expensive active surveillance programs (Aksoy et al, 2017). Vector control is also the only current method for proactively preventing people from becoming infected through tsetse fly bites. For geographically isolated regions, community-based vector control has emerged as a successful strategy (Vander et al, 2020). High resident motivation and effective application of training have demonstrated well-executed deployment with community buy-in resulting in better target care and minimized loss/damage (Vander et al, 2020). Spatial genetics
have also been integrated into vector control as a way to identify isolated populations that are ideal study sites for developing and improving vector control methods (Saarman et al, 2018).

**Disease Impact**

HAT and AAT remain as serious public health threats in sub-Saharan Africa, threatening the health and infrastructure of rural communities and agriculture in over 36 African countries (Franco et al, 2014). Not only does the disease have a high mortality rate if left untreated, but its economic and social impact is equally devastating (Franco et al, 2014).

Rural communities, where activities like agriculture, fishing, and animal husbandry expose residents to tsetse flies, bear the brunt of African Trypanosomiasis (Simarro et al, 2008). Children, women, and immunocompromised individuals are also particularly vulnerable, facing not only the debilitating effects of the disease but also the uneven disruption of livelihoods and access to education (Simarro et al, 2008). Lost productivity due to illness and death, coupled with the high cost of treatment and preventative measures, extends the impact of this disease far beyond individual health, crippling local economies and hindering community development holistically (Simarro et al, 2008). Compounding the challenges faced by these communities, limited access to adequate healthcare facilities and diagnostic tools results in delayed diagnoses and potentially fatal complications (Rocklov et al, 2020). Furthermore, the zoonotic nature of the parasite – affecting both humans and animals – leads to widespread livestock deaths, further crippling livelihoods in these already resource-limited communities (Mwiinde et al, 2022). The social impact is no less severe as chronic forms can create debilitating neurological problems or death, leaving families struggling to care for their loved ones and disrupting the social fabric of entire communities (Mwiinde et al, 2022).

**Review of Studies Relevant to the Problem: Climate Change and Vector-Parasite Interactions**

As the specter of climate change looms, understanding the climate change-driven spread and transmission intensification of vector-borne diseases is more critical than ever (Obame-Nkoghe et al, 2024). Vector-borne diseases account for 17% of the human disease burden and are projected to dramatically increase in the next decade (James et al, 2017). Changes in climate-associated host-vector-parasite dynamics hold profound implications for the public health, economic prosperity, and overall well-being of African Communities (United Nations Climate Change).

Changes in the distribution and behavior of disease-carrying vectors associated with climate change are predicted to alter the dynamics between pathogens, vectors, non-human hosts, and human populations within Africa for several diseases (Caminade et al, 2019). Vector-borne diseases such as Malaria, Dengue, Chikungunya, Rift Valley fever, Zika, and African Trypanosomiasis are all predicted to spike in prevalence in the coming decade (Mora et al, 2022). For example, field and modeling studies have shown that Anopheles mosquitoes are
migrating to higher elevations and latitudes (Caminade et al. 2014), increasing the length of malaria transmission seasons and widening the population at risk in highland regions (Carlson et al., 2023). Additionally, temperature-associated extension in habitat range due to warmer winters of the vector Ixodes ricinus infests has led to an increased prevalence of tick-borne encephalitis in northern latitudes (Gary et al, 2009). Another study conducted on female Drosophila melanogaster demonstrated that flies exposed to higher temperatures exhibited higher early fecundity but also experienced shorter lifespans, illustrating a recognized genetic trade-off (Nunney et al, Accessed 2024).

Traditionally, temperature has served as a restricting environmental factor for tsetse fly population ranges, pupation rates, and survival rates (Lord et al, 2018). Ideal environments with moderate temperatures (around 23-25°C), high relative humidity (75-90%), and shaded areas to avoid excessive evaporation have been the optimal ecosystem (Geirger et al, 2014). Rising temperatures are projected to alter the geographical distribution of both tsetse flies and trypanosomes, potentially expanding their range and increasing overlap with susceptible populations (Lord et al, 2018). A 2012 study by Moore utilized modeling to show that rising temperatures could impact Human African Trypanosomiasis epidemics. The model suggests that slight temperature increases could trigger a geographic shift in the disease, expanding the areas affected by up to 60%. This expansion could put an additional 46 to 77 million people at risk of exposure by 2090 (Moore et al, 2011).

Other climate models have echoed this prediction of expanded ranges for tsetse flies associated with increases in global temperature, particularly in areas on the fringes of traditional habitat zones. One study that used geospatial models in Northern Zimbabwe predicts a shift in tsetse populations from lower elevation to higher elevation, indicating increased susceptibility at higher altitudes (Longbottom at al, 2020). Alterations in traditional temperature regimes that previously served as barriers for tsetse fly habitation are predicted to expand into, with transitional zones between historically endemic and non-endemic regions expanding as global temperatures rise. Concurrently, there is an expected increase in the distribution of trypanosomes, with warmer temperatures speculated to provide conducive environments for proliferation (Lord et al, 2018).

Understanding the parasitology of African trypanosomes and the vector competence of tsetse flies is vital to predicting changes in transmission dynamics. The success of trypanosome transmission is intricately linked to factors such as the intrinsic incubation period, survivorship, pupa deposition, and eclosion rate in tsetse flies. However, the transmission implications for African trypanosomes and tsetse flies remain an underexplored area. The precise mechanisms through which temperature affects parasite proliferation (including survivorship and titer) and the specific influence temperature has on tsetse fly natural history traits (including fecundity, survivorship, and development rate) is still unknown.
This research project seeks to elucidate the impact of a temperature increase on the transmissibility of African trypanosomes within tsetse flies by examining effects on the life history traits of tsetse flies and their infectivity to trypanosome parasites. Understanding the potential impact of temperature on the intrinsic incubation period, survivorship, pupa deposition, and eclosion rate in tsetse flies is essential for predicting how climate change might influence the transmission dynamics of African trypanosomes. This study will quantify alterations in parasite prevalence and titer (vector competence), fecundity, female survivorship, pupa deposition, and eclosion rate between temperature-controlled flies and those exposed to an elevated temperature.

Hypotheses
This study investigates the potential effects of increasing temperatures on tsetse fly life history traits and their subsequent influence on African trypanosomiasis transmission. Specifically, the research examines how temperature variations impact fly survival, female fecundity (measured by the average number of pupae deposited per female), generation time (encompassing both time to adult emergence and gonotrophic cycle), and vector competence (prevalence of parasite infection and transmission). By analyzing these factors, the study aims to provide valuable insights into the potential consequences of climate change on African trypanosomiasis transmission dynamics and vector population demographics.

Based on previous research and established ecological principles, several hypotheses are proposed. Increased temperature is predicted to negatively impact fly longevity and reproductive output, with a decrease in both lifespan and the average number of pupae deposited per female. Similarly, a rise in temperature is expected to slow down developmental rates, leading to a delay in adult emergence and a longer time to the first gonotrophic cycle. Conversely, this study hypothesizes a potential positive influence of temperature on vector competence, suggesting that warmer temperatures might lead to a higher prevalence of parasite infection and transmission in the fly population.

2. Materials and Methods:

2.0 Insect rearing
Glossina morsitans morsitans were reared at Yale University following best practices outlined in Moloo (1971). Flies were gathered from the Yale colony at 25°C and were housed in 20 cm diameter cages within a BSL2+ insectary and maintained at 25°C, 50–60% relative humidity, and with a 14:10 hour light: dark cycle. Flies were maintained on a diet of defibrinated bovine blood provided every 48 hours through an artificial membrane feeding system and maintained for multiple generations (F0, F1, and F2), or until the line could no longer be maintained. To simulate increased temperature effects, two additional lines of flies were reared at 27°C and 28.5°C, respectively, in a designated Percival Scientific Incubator, model I-30L. To start each additional line, age-matched pregnant F0 females from the 25°Cline were obtained and
maintained in large, all-female cages with collection trays placed beneath. For all fly lines, every other day, the cages were monitored, and the number of deposited pupae and deceased adult flies were recorded. Pupae from the same generation and line were collected and pooled and transferred to an emergence cage.

2.1 Survival Analysis
To assess the mortality impact of different temperature treatments on adult flies and their progeny deposition, the survival data from pregnant females were collected. The number of dead females in pregnant female cages was recorded every other day. The proportion of live female flies was determined by subtracting the cumulative dead from the initial number of starting flies in each cage and dividing by the total initial number. These values were used to generate survival curves for each fly line and generation along with the median survival of fly line and generation using R v4.3. A horizontal line was added to the graph at the N50 value, representing the median survival time for each experimental group. The median survival in days was calculated for each temperature group by determining the time point at which the corresponding survival curve intersects the N50 line.

2.2 Generation Time and Female Fitness:
Pupa deposition and eclosion data (pupal emergence to adults) were collected to calculate generation time and female fitness. Generation time was assessed through two primary approaches: the time taken to complete the first gonotrophic cycle (first pupae deposition time) and eclosion curves depicting the time to adult eclosion from pupa deposition. For pupa deposition data, the number of pupae deposited per female, generation, fly line, and female fatalities within each pregnancy cage were recorded every other day.

In order to track the eclosion rate, the pupae deposited from pregnant female cages were transferred to transparent, individual test tubes equipped with fine-mesh emergence cages on top. Every other day, eclosion cages were checked for emergence of adult flies. When emerging flies were observed, the cage was capped and a new cage was placed on top. The capped cages with emergent adults were placed on ice to immobilize the flies and subsequently the emerging fly numbers and sex were determined. The eclosion data recorded included the number of female and male flies per cage, adult eclosion date, and generation. Finally, separate newly emerged male and female cages were set up and maintained as virgin adults. Seven days post emergence, age-matched male and female flies from the same generation and line were combined in mating cages (approximately 25 of each sex per cage) and the mating date was recorded. A week later, mated females were separated and pooled into large, all-female cages equipped with collection trays below to capture the deposited pupae representing the next generation. The remaining males from the mating cages were collected, frozen, and stored for future analysis.
Eclosion data from cages sharing the same temperature and generation were amalgamated into a unified dataset. The subsequent analysis involved creating eclosion curves and computing the mean day-to-eclosion using R and ggplot2. To ascertain the statistical significance of observed variations in eclosion times among temperature groups, an independent two-sample t-test was conducted. This test treated each temperature group (genotype) as a distinct population, comparing its mean eclosion days with that of the control 25°C group, considered as the reference. A significance level of p<0.05 was set, with any p-value below 0.05 signaling adequate evidence to reject the null hypothesis, which posited equal average eclosion times across all groups.

Female fitness was quantified by calculating the number of deposited pupae per female and the eclosion rate of those pupae to adulthood.

Average number of pupae per female=$\frac{\sum \text{Total number of pupae}}{\sum \text{Total number of females}}$

Following the calculation of the average pupae deposited per female, a one-way analysis of variance (ANOVA) was used to assess significant differences between the different temperature treatments and fly lines. If the ANOVA identified a significant difference, pairwise comparisons were performed using a post-hoc Tukey's Honestly Significant Difference (HSD) test.

2.3 Vector Competence:
Freshly emerged, teneral flies were infected with *Trypanosoma brucei brucei* strain 503 via their first blood meal two days after emergence, serving as the primary infection event. An infective blood meal was prepared by first dissolving 0.02 grams of cysteine in 100 microliters of water (technique modified from Moloo et al, 1971). Then, 50 microliters of this solution were added to 5 ml of defibrinated bovine blood, followed by a 1.5 microliter tube of the parasite (1.0 x 10^7 parasites per 1 ml of blood). This volume was sufficient to infect approximately four cages containing 25-30 flies each (Moloo et al, 1971). The infected blood was delivered through an artificial membrane feeding system.

Infection prevalence was evaluated by dissecting flies at two-time points, 21 and 28 days post-infection. The timing of dissections (days 21 and 28) aligns with the established *T. brucei* life cycle and serves to examine whether salivary gland infections could be detected earlier at higher temperatures (Silverster et al, 2017). Microscopic examination of the midgut and salivary gland organs under a dissecting microscope determined parasite presence. Dissections were performed on a SYLGARD®184 silicone matrix in a glass dish filled with phosphate-buffered saline using fine forceps. Dissected guts and salivary glands were mounted on glass slides for parasite detections.
To assess the impact varying temperature has on infection prevalence and transmission dynamics, an infection and transmission index was calculated for both gut and salivary gland infections at day 21 and day 28 for all fly lines and generations using R. The infection index is a measure of the proportion of flies, out of those that fed on an infected blood meal, that became successfully infected with the parasite. This includes both flies carrying transmissible parasites (salivary gland infections) and those with non-transmissible infections (only gut infections). The transmission index reflects the proportion of infected flies carrying transmissible parasites (parasites present in the salivary glands) relative to the total population that is infected (flies with gut infections that are not transmissible). The transmission index is used to assess the efficiency of parasite transmission to a new host (Telleria-El et al., 2014). The transmission and infection index was calculated using the following equations below.

Transmission Index: \( \frac{\Sigma(SG)}{\Sigma(SG+GUT)} \)

Infection index: \( \frac{\Sigma(SG+GUT)}{\Sigma(SG+GUT+NEG)} \) (for each treatment)

3. Results:
3.0. Survival Analysis
The 25°C fly line (denoted as Wt-Amb25°C) had the longest survival time, with nearly all flies surviving until approximately day 60 (Table 1; Figure 1). Following day 60, an acute mortality phase occurred where survival rates dropped starkly. This fly line had the longest median survival time out of all tested temperatures, around 66 days. Once the survival decline occurred, the Wt-Amb25°C’s acute mortality phase’s curve matched those of the F0_28.5°C and F1_28.5°C fly line’s curve.

<table>
<thead>
<tr>
<th>Fly Line and Generation</th>
<th>Survival Time</th>
</tr>
</thead>
<tbody>
<tr>
<td>Wt-Amb25°C</td>
<td>66</td>
</tr>
<tr>
<td>F0_27°C</td>
<td>56</td>
</tr>
<tr>
<td>F0_28.5°C</td>
<td>28</td>
</tr>
<tr>
<td>F1_28.5°C</td>
<td>16</td>
</tr>
</tbody>
</table>

Table 1: Median survival time in days for female flies reared at three temperatures (25°C, 27°C, 28.5°C) across generations. The "wt" group denotes the wild-type flies kept at the ambient temperature (25°C). The data reveals a negative correlation between temperature and median female fly longevity. Notably, relative to flies at Wt-Amb25°C, flies reared at the highest temperature (28.5°C) displayed shorter lifespans with the F1_28.5°C fly line (progeny of the F0_28.5°C) having the most dramatic negative impact on fly survivorship.
Figure 1: Survival of female flies reared at three temperatures (25°C, 27°C, and 28.5°C) across generations. The surviving female flies were tracked every other day and the proportion alive was determined by subtracting the cumulative deaths over time from the starting number of flies in each cage and dividing by the initial population per cage. Time is measured in days since adult emergence from the pupa. The survival curve for the Wt-Amb25°C fly line displays the longest stable survival, with nearly all flies surviving until approximately day 60. The F0_27°C survival curve exhibits a gradual but consistent decrease in survival throughout the 60-day experiment. The 28.5°C fly line includes two curves, F0 (the first generation) and F1 (the offspring of the F0 generation). The F0_28.5°C generation’s curve maintained a stable survival rate until day 17, at which point a sharp decline in survival occurred. The F1_28.5°C generation experienced the most precipitous decline in survival, with a sharp drop around days 5-6 that persisted.
The F0_27°C flies survival exhibits a gradual and consistent rate of mortality with a median survival time of 56 days. This is shorter than the Wt-Amb25°C fly line but considerably longer than both 28.5°C generations. The F0_28.5°C generation maintained a stable survival rate until day 17, followed by a sharp decline in survival and a median survival time of 28 days. The F1_28.5°C generation experiences the most precipitous decline in survival with the sharpest drop occurring around day 5-6 (more than 10 days before the previous generation and ~55 days before the control group). With a median survival of just 16 days, these flies exhibit the sharpest and most sustained acute mortality phase. Despite the initial sharp decline, the acute mortality phase begins to level off after day 20, resembling the more gradual decrease in survival observed in the F0_27°C flies. The F1_28.5°C generation displayed a higher proportion of survival at day 40 (approximately 12.5%) compared to the F0_28.5°C generation (7.25%).

3.1 Generation Time:
The range for the time to eclosion for all temperature groups was between 12 and 32 days (Figure 2, Table 2). The Wt-Amb25°C fly line displayed the slowest development, with flies emerging from pupal development between days 23 and 32 (10-day window) with an average eclosion time of 29.25 days. While the Wt-Amb25°C fly line exhibited the longest overall development time among the tested temperatures, it displayed the steepest eclosion curve with a slope of 0.10. This indicates a faster rate of adult emergence once eclosion begins compared to other temperature treatments (Table 3).

Table 2: Mean days to adult eclosion from flies reared at three temperatures (25°C, 27°C, 28.5°C) across generations. At the highest temperature tested (28.5°C), both the F0 and F1 fly lines had the fastest average time to adult eclosion. In contrast, the Wt-Amb25°C fly line displayed the longest time to adult eclosion and the 27°C fly line emerged at an intermediate time point in between the other fly line’s mean eclosion days.

<table>
<thead>
<tr>
<th>Fly Line and Generation</th>
<th>Mean Days to Adult Eclosion</th>
</tr>
</thead>
<tbody>
<tr>
<td>Wt-Amb25°C</td>
<td>29.05</td>
</tr>
<tr>
<td>F0_27°C</td>
<td>23.05</td>
</tr>
<tr>
<td>F0_28.5°C</td>
<td>21.54</td>
</tr>
<tr>
<td>F1_28.5°C</td>
<td>21.90</td>
</tr>
</tbody>
</table>
Figure 2. Eclosion curve depicts the proportion of eclosed flies across different temperatures and generations over time. The x-axis represents days from pupation to adult eclosion, while the y-axis indicates the proportion of flies that have eclosed. A dashed line at 50% eclosion serves as a reference point, indicating when half of the population has eclosed. All elevated temperature curves are shifted leftward relative to Wt-Amb25°C, indicating sooner fly emergence at higher temperatures. The time for 50% of each fly line’s flies to eclose was 22 days for both generations reared at 28°C and 23 days for F0_27°C flies.
The 28.5°C fly line exhibits the earliest adult eclosion timing, with emergence starting as early as day 13. The starting eclosion day for the F0_28.5°C fly line was day 13 and F1_28.5°C was day 14 and the mean day to eclosion for both was 21 days. The time for 50% of each fly line’s flies to eclose was 22 days for both generations. The slope for F0_28.5°C was 0.070 and the slope for F1_28.5°C was 0.0746 (Table 3). The 27°C fly line began to eclose at day 17, 5 days later than the 28.5°C fly lines. The slope for the F0_27°C flies was 0.0718.

To determine if the observed differences in eclosion times between the elevated temperature fly lines compared to the Wt-Amb25°C fly line were statistically significant, independent two-sample t-tests were performed (Table 4). The results showed that the F0_27°C fly line eclosed significantly earlier than the Wt-Amb25°C control group (independent two-sample t-tests, p=1.7e-19) as did F0_28.5°C and F1_28.5°C (p=2.8e-10 and p=1.3e-10 respectively).

Table 3: Slopes of the eclosion curves for three temperatures (25°C, 27°C, and 28.5°C) across generations. Each slope value represents the rate of change in the proportion of eclosed adults over time, measured in days from pupation to adult eclosion. The Wt-Amb25°C exhibits a relatively larger slope compared to other fly lines, indicating a faster rate of eclosion at 25°C.

<table>
<thead>
<tr>
<th>Fly Line</th>
<th>Eclosion Slope</th>
</tr>
</thead>
<tbody>
<tr>
<td>Wt-Amb25°C</td>
<td>0.101</td>
</tr>
<tr>
<td>F0_27°C</td>
<td>0.072</td>
</tr>
<tr>
<td>F0_28.5°C</td>
<td>0.070</td>
</tr>
<tr>
<td>F1_28.5°C</td>
<td>0.075</td>
</tr>
</tbody>
</table>
Table 4: Independent two-sample t-tests. Assessed the statistical significance of differences in eclosion times between flies reared at increased temperatures compared to the Wt-Amb25°C (27°C and 28.5°C) across generations. The analysis includes three fly lines and generations (27°C, F0_28.5, and F1_28.5) compared to the Wt-Amb25°C control fly line. All three fly lines exhibit statistically significant differences in mean days to eclosion relative to the Wt-Amb25°C fly line.

<table>
<thead>
<tr>
<th>Fly Line</th>
<th>Eclosion Day P-values</th>
</tr>
</thead>
<tbody>
<tr>
<td>F0_27°C</td>
<td>1.7e-19</td>
</tr>
<tr>
<td>F0_28.5°C</td>
<td>2.8e-10</td>
</tr>
<tr>
<td>F1_28.5°C</td>
<td>1.3e-10</td>
</tr>
</tbody>
</table>

Mated females from the Wt-Amb25°C fly line exhibited a gonotrophic cycle initiation time of 27 days, which was the longest among the fly lines studied. In contrast, the F0_27°C fly line demonstrated a faster initiation, taking only 22 days to commence the first gonotrophic cycle. The F0_28.5°C fly line showed a more accelerated process, with the cycle initiating in just 14 days. The F1_28.8°C fly line had the quickest initiation time at merely 12 days.

3.2 Female Fitness:
Females in the Wt-Amb25°C control group displayed the highest reproductive output, depositing an average of 4.75 pupae per female (Table 5). This was followed by F0_27°C flies depositing an average of 3.26 pupae per female and then the F0_28.5°C line that deposited an average of 1.85 pupa per female. Females of F1_28.5°C fly lines had the lowest fecundity with an average pupae deposition at less than 1 pupae per female (0.47).
Table 5. Average pupae deposition per female flies reared at three temperatures (25°C, 27°C, 28.5°C) across generations. Female fitness was assessed by calculating the number of pupae per female. The formula used to calculate the average number of pupae per female is: Average number of pupae per female = \( \frac{\text{Total number of pupae}}{\text{Total number of females}} \).

<table>
<thead>
<tr>
<th>Fly Line</th>
<th>Total Pupae</th>
<th>Number of Total Females</th>
<th>Number of Average number of pupae per female</th>
</tr>
</thead>
<tbody>
<tr>
<td>Wt-Amb25°C</td>
<td>380</td>
<td>80</td>
<td>4.75</td>
</tr>
<tr>
<td>F0_27°C</td>
<td>1141</td>
<td>350</td>
<td>3.26</td>
</tr>
<tr>
<td>F0_28.5°C</td>
<td>427</td>
<td>231</td>
<td>1.85</td>
</tr>
<tr>
<td>F1_28.5°C</td>
<td>72</td>
<td>152</td>
<td>0.47</td>
</tr>
</tbody>
</table>

In order to test if the pupa deposition data was normally distributed, the Shapiro-Wilk test for normality using the significance of \( p > 0.05 \) was done. The output was 0.97, indicating that the data was normally distributed. In order to test if these differences in average number of pupae per female were significant, an ANOVA found significant differences between the groups (ANOVA \( P=0.03 \)), indicating significant variations in the average number of pupae deposited per female across the temperature groups and generations. Subsequent Tukey's (HSD) post-hoc test elucidated specific pairwise differences. While there was no significant difference observed between the F0_27°C group and the control group (wt_Amb25) \( (p = 0.45) \), both the F0_28.5°C and F1_28.5°C groups exhibited significantly different average pupae deposition compared to the control group \( (p = 0.001 \text{ and } p = 0.02, \text{ respectively}) \). There was no significant difference observed between the F0_27°C group and the control group (wt_Amb25) \( (p = 0.58) \). These results suggest that while the 28.5°C temperature groups exhibit significant differences in pupae deposition compared to the control, the 27°C temperature group does not.

3.3 Vector Competence:
The Wt_Amb25°C fly line displayed an infection index of 0.816, alongside a transmission index of 0.512 on day 21 (Table 7, Table 8). This suggests that 82% of all dissected flies carried infections (salivary gland plus midguts), with 51% of those infected carrying transmissible salivary gland infections. For Wt-Amb25°C flies, the transmission index decreased only by 1% and the infection index by 5% at day 21 relative to day 28. In contrast, flies reared at 27°C showed the highest infection indices of 0.90 and 0.88 on days 21 and 28, respectively. Interestingly, the transmission index for flies reared at 27°C increased over time, from 0.39 on...
Flies reared at 28.5 °C had an infection index of 0.78 on day 21, slightly rising to 0.79 by day 28, which was the smallest variation in infection index noted among all the temperature groups. However, the transmission index saw a significant drop from 0.53 on day 21 to 0.35 on day 28 (a 19% decline in transmission index), representing the most substantial decrease in transmission index observed across the study. This inconsistency could arise from either excessive infected fly deaths during this period and/or the small number of remaining adults screened.

Table 6: Infection Index Calculation Across Temperature Groups. This table presents the infection index calculated as $\Sigma(SG+GUT) / \Sigma(SG+GUT+NEG)$ for each treatment group. Infection rates for both gut and salivary gland infections were assessed at 21 and 28 days post-infection using microscopic examination of dissected flies. The dissections were performed at two time points, 21 and 28 days post-infection, with the parasite presence determined through microscopic examination of midgut and salivary glands. All temperature treatments were evaluated for infection rates at both time points. At day 21 and 28 post-infection, flies reared at 27 had the highest infection index. Flies reared at 28.5°C experienced the smallest change in infection index from day 21 to day 28 (1% increase) and flies reared at 25°C experienced a drop of 5% in infection index from day 21 to day 28.

<table>
<thead>
<tr>
<th>Fly Line</th>
<th>Day Infection</th>
<th>Post Infection</th>
<th>Total Number of</th>
<th>Total Number of</th>
<th>Infection Index</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Salivary Gland</td>
<td>Gut Infections</td>
<td></td>
</tr>
<tr>
<td>WT_Amb25°C</td>
<td>21</td>
<td>84</td>
<td>103</td>
<td>0.82</td>
<td></td>
</tr>
<tr>
<td>WT_Amb25°C</td>
<td>28</td>
<td>56</td>
<td>73</td>
<td>0.77</td>
<td></td>
</tr>
<tr>
<td>Temp_27°C</td>
<td>21</td>
<td>18</td>
<td>20</td>
<td>0.90</td>
<td></td>
</tr>
<tr>
<td>Temp_27°C</td>
<td>28</td>
<td>14</td>
<td>16</td>
<td>0.88</td>
<td></td>
</tr>
<tr>
<td>Temp_28.5°C</td>
<td>21</td>
<td>39</td>
<td>50</td>
<td>0.78</td>
<td></td>
</tr>
<tr>
<td>Temp_28.5°C</td>
<td>28</td>
<td>34</td>
<td>43</td>
<td>0.79</td>
<td></td>
</tr>
</tbody>
</table>
Table 7: Transmission index calculation for flies reared at 25°C, 27°C, and 28.5°C. This was calculated using the equation: $\Sigma(SG) / \Sigma(SG+GUT)$. Transmission indexes were calculated at day 21 and day 28. The highest transmission index was observed in flies reared at 27°C on day 28 and this temperature group experienced an increase in transmission index over time. The transmission index decreased by .1 over time for Wt-Amb25 and went down by .19 over time for 28.5°C flies.

<table>
<thead>
<tr>
<th>Fly Line</th>
<th>Days Infection</th>
<th>Post Infection</th>
<th>Total Number of Salivary Gland and Gut Infections</th>
<th>Number of Salivary Gland Infections</th>
<th>Transmission Index</th>
</tr>
</thead>
<tbody>
<tr>
<td>Wt-Amb25°C</td>
<td>21</td>
<td>84</td>
<td>43</td>
<td>.51</td>
<td></td>
</tr>
<tr>
<td>Wt-Amb25°C</td>
<td>28</td>
<td>56</td>
<td>28</td>
<td>.50</td>
<td></td>
</tr>
<tr>
<td>Temp_27°C</td>
<td>21</td>
<td>18</td>
<td>7</td>
<td>.39</td>
<td></td>
</tr>
<tr>
<td>Temp_27°C</td>
<td>28</td>
<td>14</td>
<td>8</td>
<td>.57</td>
<td></td>
</tr>
<tr>
<td>Temp_28.5°C</td>
<td>21</td>
<td>39</td>
<td>21</td>
<td>.54</td>
<td></td>
</tr>
<tr>
<td>Temp_28.5°C</td>
<td>28</td>
<td>34</td>
<td>12</td>
<td>.35</td>
<td></td>
</tr>
</tbody>
</table>
Discussion:
This study investigated the potential impact of increased temperature on tsetse fly life history traits and their influence on African Trypanosome infections. This was done in order to elucidate how increased temperatures could shift key tsetse fitness parameters, including fecundity, longevity, eclosion timelines, maturation rate, and vector competence (parasite infection/transmission prevalence). As hypothesized, increased temperatures significantly affected fly survival, development, and potentially, vector competence (transmission of the parasite).

Consistent with the hypothesis, higher temperatures negatively impacted fly longevity. The decrease in the median survival times and the observed higher acute mortality in temperature treated flies suggests a pronounced reduction in survivorship at higher temperatures, with the most stark survival decline noted in the F1 generation from the 28.5°C fly line. Interestingly, flies reared at 27°C displayed a lower mortality rate compared to 28.5°C fly lines, demonstrating that higher temperatures lead to higher mortality rates.

Contrasting our initial hypothesis, which predicted a negative correlation between temperature and tsetse development, the observed data revealed an acceleration in tsetse maturation across the temperature treatments. Earlier pupal deposition and adult emergence were observed across higher temperature treatments, indicating a temperature-induced effect on maturation. However, this appeared to come at the cost of reduced fecundity as a negative correlation between temperature and the mean number of reproductive outputs was found. This suggests a potential decline in reproductive fitness at higher temperatures, which is in line with what was originally hypothesized. Notably, the F1_28.5°C group displayed the most substantial decrease in fecundity, potentially indicating a critical temperature limit for reproductive viability at higher temperatures.

Infection dynamics results presented a complex picture. The 27°C group exhibited a rising infection index from day 21 to day 28 and the 28°C fly line had the most infections at early time points. However, the 28.5°C group contracted this trend as their infection index remained constant and their transmission index decreased from day 21 to day 28. Notably, the observed decrease in fly longevity at higher temperatures may have skewed infection indexes. Shorter lifespans could have led to underestimation of infection prevalence as the infected flies died before dissection, or infection peaked earlier and was missed by our dissection time points. Future studies with earlier dissection time points (e.g., day 14) could address this limitation.

To summarize the observed fitness decline under thermal stress raises concerns about potential fly population collapse if adaptation is not possible. This experiment utilized a relatively small starting population, and future studies with larger populations might reveal a greater capacity for adaptation. Additionally, the use of laboratory-reared colonies, which lack the genetic diversity
of wild populations, could underestimate their ability to adapt to changing temperatures (El Yamliali et al, 2023). If adaptation does occur, it could significantly alter the trypanosomiasis-tsetse fly dynamic. Changes in vector competence and parasite incubation duration are potential consequences of thermal adaptation in the fly population. Ultimately, predicting the long-term evolutionary consequences of rising temperatures is complex, but this study provides a valuable starting point for understanding these potential effects.

Vector Competence:
Infection and transmission dynamics exhibited complex results across temperature treatments. The 27°C fly line displayed consistently high infection indexes at both day 21 and day 28, while the other temperature treated fly lines (25°C and 28.5°C) showed declines. Transmission indexes also varied; flies at 25°C maintained a stable value, whereas the 27°C fly line increased and the 28.5°C fly line decreased in value from day 21 to day 28.

Two potential factors might explain these contrasting results, particularly the decline in transmission observed in the 28.5°C group. The first is differential mortality rates between infected flies and noninfected flies. Previous studies suggest that tsetse flies infected with the parasites are more susceptible to mortality compared to uninfected flies (Maudlin et al., 2011). Uninfected flies are more resilient and more likely to survive long enough to undergo dissection and infection testing than infected flies (Akoda et al., 2009). This underscores a potential bias in the findings as infected flies that died before dissections likely went undetected, leading to an underestimation of true infection prevalence. Consequently, the reported indexes may not accurately reflect the true prevalence of infection, as uninfected flies dominate the surviving population subjected to analysis, skewing the calculated infection and transmission indexes. The substantial mortality difference between infected and uninfected flies, particularly at higher temperatures, emerged as a major limitation of this study. Based on these results, future studies should address the limitation of differential mortality by incorporating earlier dissection time points, such as day 14. This would help capture infected flies before mortality significantly impacts the sample pool, leading to a more accurate estimation of infection prevalence and transmission dynamics. Additionally, exploring even earlier time points could provide valuable insights into the initial establishment and progression of salivary gland infections at different temperatures. This would help elucidate whether higher temperatures directly influence the parasite's ability to establish infection in the fly.

The second factor impacting the vector competence results is limited sample size in the temperature treated flies. While it was hypothesized that temperature would negatively impact fly longevity, the amount of mortality that occurred in temperature treated flies far exceeded expectations. The result was a drastically reduced sample pool for temperature treated flies. The decrease in fly survival at higher temperatures substantially reduced the pool of available flies for dissection at day 21 and 28 compared to the temperature controlled fly line. As a result, the
transmission indexes of infected flies at higher temperatures have skewed representations of the remaining population’s infected prevalence. This limited sample size, particularly evident in the 27°C group (18 flies on day 21 and 14 flies on day 28) introduces challenges in drawing definitive conclusions about transmission dynamics. Due to these results, moving forward it would be necessary to increase the starting population size for flies reared in increased temperatures in order to account for the large crash in population numbers associated with temperature.

**Generation time:**
Eclosion time exhibited a significant positive response to temperature, contradicting the initial hypothesis of a slower emergence rate at higher temperatures. Flies reared in warmer treatments emerged considerably earlier than the control fly line. Notably, flies reared at 28°C displayed the fastest emergence, closely followed by the 27°C fly line. Interestingly, flies reared at the control temperature had an eclosion slope that was steeper compared to the heat-treated fly lines, suggesting a shorter window of emergence once eclosion did occur. The slopes within the heat-treated fly lines themselves were quite similar and generations reared at the same temperature had practically identical slopes.

There are a few possible explanations for the observed results. First, temperature plays a role in accelerating the developmental process of flies, leading to quicker maturation and emergence. Elevated temperatures could function as a catalyst, expediting the stages of growth and development in flies. This would explain why flies subjected to higher temperatures may mature and emerge as adults more swiftly compared to those experiencing lower temperature conditions. This explanation finds support in other research studies where temperature has been identified as a factor that accelerates development in various fly species. For example, Sukiato et al. (2019) observed a significant reduction in developmental time (hatching to adult emergence) in Aedes aegypti mosquitoes with increasing temperatures. Similarly, Bambaradeniya et al. (2019) reported the fastest development of Chrysomya megacephala at the highest tested temperature of 38°C compared to lower temperatures. Additionally, another study conducted on Drosophila melanogaster found high temperature stress had negative effects on the developmental durations of D. melanogaster (Hu D et al, 2023). Furthermore, another study found the number of days required for adult emergence decreased with increase in temperature for Bactrocera carambolae and Bactrocera papayae (fruit flies) (Danjuma et al, 2014).

These findings suggest that regions experiencing rising temperatures due to climate change may witness a more rapid maturation and emergence of tsetse fly populations. This accelerated eclosion could lead to larger adult tsetse fly populations in warmer regions. Such a scenario, where the vector population is primarily composed of mature adults, could exacerbate disease transmission. Mature adult vectors are known to be more efficient at transmitting pathogens, potentially due to higher pathogen loads resulting from longer exposure times and increased
feeding activity (Geiger et al, 2014). This heightened efficiency may lead to increased disease transmission rates within the population. Additionally, the shorter incubation period facilitated by a higher proportion of mature adults within a region could result in more rapid disease outbreaks and heightened transmission intensity within affected communities (Aksoy et al, 2017). Furthermore, the increased emergence rate of mature adults may pose challenges for vector control efforts. Mature adult vectors are often more resilient to control measures such as insecticides or environmental management strategies, making them difficult targets for control programs (Aksoy et al, 2017). Therefore, the accelerated emergence of tsetse flies could potentially complicate existing vector control initiatives, necessitating careful consideration of timing and strategies in control programs.

Female Fitness:
Our findings revealed significant differences in fecundity across the temperature treatments, supporting the hypothesis that increased temperature decreases reproductive outputs. The Wt-Amb25°C control fly line exhibited the highest average pupae deposition per female, consistent with expectations of 25°C serving as the optimal temperature for reproductive performance. Conversely, the F0_28.5°C and F1_28.5°C fly lines displayed flies depositing fewer pupa, indicating reduced reproductive fitness under elevated temperatures. This observation suggests the possibility that 27°C may represent a suboptimal temperature for tsetse fly reproduction. These findings suggest that a temperature of 27°C does not have a noticeable effect on reproductive output, whereas higher temperatures, such as 28.5°C, represent a threshold temperature that impacts fecundity.

The results of temperature negatively influencing fecundity is reported in other studies. For example, a study examining Bactrocera dorsalis found reproduction was negatively affected through less reproductive outputs during high-temperature stresses, reaching the lowest value at 36 °C (Yu C et al, 2022)

From a transmission perspective, these results imply that modest increases in temperature (seen through flies reared at 27°C) may not act as a limiting factor for tsetse fly fecundity. The lack of statistically significant differences in reproductive output between flies reared at 27°C and the temperature control group implies under scenarios of global warming where temperatures increase by a few degrees, tsetse fly populations will remain stable due to the relatively unchanged number of pupae deposited. This resilience in reproductive fitness underscores the potential for stable vector populations to sustain disease transmission cycles despite gradual changes in temperature over time.

Moving forward, future research should focus on elucidating the specific physiological and molecular mechanisms underlying the observed differences in pupa deposition across temperature treatments. For instance, investigating the hormonal regulation of reproductive
processes in response to temperature fluctuations could provide valuable insights into the molecular pathways involved. Additionally, exploring the genetic basis of temperature-mediated changes in reproductive fitness, particularly genes associated with developmental timing and fecundity, could offer further understanding. Conducting experiments to manipulate these pathways or genes in controlled settings may help validate their role in temperature-induced changes in reproductive output. Furthermore, studying the interactions between temperature and other environmental factors, such as humidity or nutrient availability, could provide a more comprehensive understanding of tsetse fly population dynamics.

**Survival analysis:**
Survival analysis revealed the Wt-Amb25°C line had the highest longevity, with most flies living up to day 60. In contrast, the F0_27°C line showed a steady, gradual decline in survival. The 28°C lines exhibited varied patterns: F0_28.5°C had stable survival until day 17 before declining sharply, while the F1_28.5°C saw a steep drop in survival early on, around days 5-6.

When analyzing the gradual decline in survivability evident in the 27°C fly line relative to the more abrupt drop in mortality observed in other temperature-treated lines, a plausible explanation could involve a chronically elevated stress response in these flies. This response, while not sufficiently severe to cause immediate mortality as seen in the 28.5°C temperature-treated flies, could be a contributing factor to their gradual decline. Stress response pathways could be diverting resources away from vital physiological processes (such as immune activity or metabolism), ultimately shortening lifespan. As an example, Heat Shock Protein (HSP) response has been shown to be activated by high temperatures in gall flies (*Procecidochares utilis*) in order to protect fly cells from temperature-related cellular damage (Liang et al, 2023). Similar results are echoed in other studies, such as Cavieres et al. (2020), which examined the relationship between thermal conditions on temperature tolerance and demographic parameters in *D. melanogaster*. This study found that high parental thermal environments lowered survival, fecundity, and net reproductive rate.

Another possible explanation for the differences in survival between generation’s reared at the same temperature could be due to transgenerational epigenetic effects influencing offspring’s lifespan and adaptations. Epigenetics encompasses alterations in gene expression mediated by mechanisms that do not involve changes in the underlying DNA sequence (Handy et al., 2011) Transgenerational epigenetic marks can occur when parental exposure to environmental factors (such as temperature) alters the epigenetic landscape of the germline, which impacts the offspring’s gene expression, development, and health(Heard et al, 2014). These epigenetic effects have been explored and documented in other fly species as a factor influencing longevity and development. For example, one study examining the epigenetic responses of fruit flies to toxic stress resulted in developmental changes that increased flies tolerance to typically lethal toxic concentrations over time (Stern et al, 2012). These epigenetic changes were observed to be
transgenerationally inherited, with offspring inheriting the genetic adaptations without direct exposure to the toxin. This illustrates that transgenerational epigenetic effects can manifest in flies, influencing offspring survival in response to environmental stresses.

**Conclusion:**
This study investigated the impact of temperature variations on tsetse fly life history traits and their potential influence on African trypanosome infections. Our findings highlight a complex interplay between temperature and various aspects of tsetse fly biology. Increased temperatures resulted in faster developmental times but reduced fly longevity, particularly at the highest temperature tested (28.5°C). Additionally, female fecundity declined at elevated temperatures, suggesting a potential negative impact on reproductive fitness. Interestingly, a modest temperature increase (27°C) did not significantly affect fecundity, indicating a possible threshold effect for a new suboptimal temperature.

While vector competence, as measured by infection and transmission indexes, was not definitively established due to mortality bias at higher temperatures, our results suggest potential complexities in disease dynamics under these temperature conditions and a need to check earlier timepoints to see if trypanosomes are transmissible earlier. Notably, the observed decrease in fly survival at higher temperatures may limit the overall lifespan of infected individuals, potentially reducing their contribution to disease transmission. However, in wild populations the potential for adaptation to these elevated temperatures cannot be ruled out, warranting further investigation.

Overall, this study underscores the multi-factorial effects of temperature on tsetse fly biology and disease transmission dynamics. Our findings provide valuable insights for developing effective control strategies for tsetse-borne African trypanosomiasis in a changing climate. Future research should delve deeper into the underlying mechanisms driving the observed effects and explore potential intervention strategies targeting these mechanisms to mitigate disease risks under scenarios of global warming.
References


