Survivorship of Anopheles gambiae sensu lato in irrigated sugarcane plantation scheme in Ethiopia

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Abstract

Background: To ensure food security, the sub-Saharan Africa has initiated massive water resource development projects, like irrigated agriculture, in recent years. However, such environmental modifications affect the survivorship and development of mosquitoes that are vectors of different diseases. This study aimed at determining the effects of irrigation practices on development and survivorship of *Anopheles gambiae* s.l in Ethiopia.

Methods: Life-table experiment was conducted to examine the effect of environmental modification on survivorship of both immature and adults *An. gambiae s.l.* in irrigated and non-irrigated areas. The pupation rate and development time of the immatures and adult longevity and fecundity were compared between the two settings.

Results: The estimated mean survival time of female *An. gambiae s.l.* in irrigated and non-irrigated area was 37.9 and 31.3 days, respectively. A survival analysis showed that adult females of *An. gambiae s.l.* placed in irrigated area lived significantly longer than those at the non-irrigated area ($\chi 2 = 18.3$, df = 1, $p < 0.001$) and *An. gambiae s.l.* females lived significantly longer than males in both areas ($p < 0.001$).

Conclusions: Adult *An. gambiae s.l.* survivorship was found to be enhanced in irrigated area compared to non-irrigated area. Longer survival of adult mosquito in irrigated area could have important implications in vectorial capacity and hence malaria transmission.

Background

Mosquito survivorship is an important factors that determine vectorial capacity, and malaria transmission potential [1]. For example, *Anopheles* mosquito needs to survive beyond the extrinsic incubation period of the *Plasmodium* parasites to be able to transmit malaria; the longer a mosquito lives the higher the number of bites it may causes [2]. Malaria vector immatures survivorship and enhanced larval-to-pupal development rate increase adult population density, which in turn affect vectorial capacity of mosquito populations in a particular setting [3, 4].

Mosquito survivorship and development may be affected by environmental factors. Temperature (both water and ambient), relative humidity, rainfall, nutrient availability are key environmental factors governing the dynamics of malaria vectors including development and survival [4–6]. These factors can strongly be influenced by variation in land use and land cover change such as vegetation cover, landscape, distance to water body [7,8]. Zhong *et al* [9] and Wang *et al* [10] reported enhanced survivorship and development of both adult and larve of *An. sinensis* and *An. minimus,* major malaria vectors in China with higher ambient temperature due to land use and land cover change. Fine-scale variation in microclimate across different landscapes shapes variation in mosquito population dynamics [11].

In effort to avert poverty, developing countries have been implementing water resource development projects like hydropower dams and agricultural development irrigation schemes [7,12,13]. Previous studies indicated that such change in land use and land cover has been increased malaria transmission through proliferating vector breeding sites and changing the microclimate that governs the dynamics of the vectors [7,13–19].

Ethiopia, a country with more than 75 % of the total area is malarious [20], has been experiencing a massive change in land use and land cover through water resource development projects including irrigation schemes, and hydroelectric power dam projects [21]. Arjo-Dedessa sugar development project site is among mega irrigation schemes with a irrigated covering around 4,000 ha. with future expansion plan of 80,000 ha, which supplies a state-owned sugar factory [22]. The area is historically known to be a wildlife sanctuary. Long ago, the government, partly, settled residents evacuating from other draught prone areas of the country to establish their lives through subsistence farming. The area is endemic to malaria [22]. A recent entomological study in the same study site demonstrated higher malaria vector breeding habitat diversity, larval occurrence and abundance in the irrigated area than the non-irrigated area [23]. However, how this massive environmental modification has been influencing the survivorship and development of major malaria vector in the area is not yet understood. Understanding of malaria vector bionomics in line with environmental modification helps to model malaria transmission for better evidence-based interventions, which will have a profound effect in realizing the country's malaria elimination goal by 2030 [24]. We hypothesized that land use and land cover changes, especially massive irrigated agriculture alter the survivorship and development of malaria vectors in the areas.

Therefore, the objective of this study was to determine the effects of irrigated sugarcane plantation scheme on development and survivorship of *An. gambiae s.l.* Knowledge of vector response to environmental modification will give a better understanding of malaria transmission dynamics, which is useful for predicting the impact of environmental modification on malaria transmission intensity and set forward tailored vector control interventions.

Methods

Study setting and period

The study was conducted in Arjo-Dedessa irrigation development site (8° 41' 60" N, 36° 23' 60" E), Southwest Ethiopia, from August to October 2019. Extensive irrigated agriculture represents the most important environmental change in the area. The irrigation development areas were covered with a massive irrigated sugarcane plantation (irrigated area, hereafter), whereas the surrounding areas were covered with other non-irrigated field crops commonly cultivated in the area (non-irrigated area, hereafter). Local communities in the area depend on subsistence farming whereby practicing smallholder non-irrigated cultivation of mixed crops and cereals. The common crops and fruit trees grown in the area include corn, maize, peanut, sorghum, rice, wheat, coffee and mango.

Site selection

For the study, we selected two land use and land cover types: areas covered with irrigated sugarcane plantation area and areas covered with other field crops common in the area.

Anopheles gambiae *s.l.* immature survivorship

Adult mosquito collection and larvae hatching: Blood-engorged *An. gambiae s.l.* were collected from inside houses and animal shelters in the study area using mouth aspirator. All collected mosquitoes were kept in paper cages at field insectary. An oviposition substrate with Petri-dish lined with a filter paper disk on moistened cotton wool kept inside each cage for egg laying. Collected eggs were allowed to hatch and newly hatched first instar larvae were used for the experiment.

Experiment: Plastic washbasin (34 cm x 14 cm) was used to imitate natural larval breeding habitat. The washbasins were exposed to outdoor environment for a week prior to the initiation of experiment for acclimatization. Then, two liters of rainwater and 1 kg of soil from the same area was added in each washbasin and left for a day. The washbasins were kept at each selected site in the two different areas (irrigated and non-irrigated areas). Fifty newly emerged first instar *An. gambiae s.l.* larvae were transferred into each washbasin with eight replicates for each sites. The water level in the washbasins was checked daily and maintained by adding water if needed. To prevent other insects from invading the washbasins or other mosquitoes from laying eggs, the washbasins were placed inside an insect-proof 61 × 61 × 61 cm^3 BugDorm tent (BioQuip, Rancho Dominguez, (BD2120), CA, USA) (Fig. 1). All sides of the BugDorm tent were made of clear polyester netting materials, so that sunlight was not blocked. The homogeneity of washbasins had the advantage over the natural habitats, which were highly variable in habitat size, larval food conditions (e.g. organic matters), vegetation coverage, light shade, competitors and predators. Each day the number of surviving larvae and their developmental stage, and mortality was recorded. Pupae were counted and removed daily. Removed pupae were collected in the waterproof paper cup for adult emergence.

Anopheles gambiae s.l. adult survivorship experiment

In this experiment, *An. gambiae s.l.* adults emerged from the larval survivorship experiments were used. Twenty-five female and 25 male adult mosquitoes within 24 hours post-emergence were transferred into a paper cage (21.5 cm x 9 cm). The cages were covered with nylon mesh to prevent mosquito escape. Then, the cages were placed at irrigated and non-irrigated area in five replicates for each site. Mosquito cages were hanged from the roof structures of small temporary shelters (2m high) constructed for the experiment purpose for rain protection (Fig. 2). To prevent ants from reaching and scavenging on mosquitoes, grease was applied to the suspension twines. Mosquitoes were provided with 10% sucrose solution and blood meal every other day for 20 minutes from human arm (DH). An oviposition substrate consisting of a Petri-dish lined with filter paper disk on moistened cotton wool was placed for egg-laying. An oviposition substrate in each cage was examined daily for the presence of eggs and the number of eggs laid was examined under a dissecting microscope counted and recorded. The cages were examined

daily for the numbers of surviving and dead mosquitoes. Then, the dead mosquitoes were recorded and removed from the cage daily.

Microclimate data collection

For larval survivorship experiment, HOBO data logger (Onset Computer Corp., MX2202, Bourne, MA) was placed in each washbasin, 1 cm below the water surface and then hourly water temperature and light intensity were recorded for the entire experiment duration. For adult survivorship experiment, HOBO data loggers (Onset Computer Corp., MX2301, Bourne, MA) was kept nearby the experiment setup at 2 m above ground and then the hourly ambient temperature and relative humidity were recorded during entire duration of the experiment.

Data analysis

The pupation rate of *An. gambiae s.l.* larvae was calculated as the proportion of first instar larvae that developed into pupae. Mean larval-to-pupal development time was calculated. Kaplan-Meier survival analysis was performed to determine the variation in mean daily survivorship of mosquitoes placed in two different land use and land cover areas. A log-rank test was used to determine the difference between two survival curves. Daily average, minimum, and maximum temperatures, relative humidity and light intensity were calculated from the hourly record data to determine the effect of different land use and land cover on the microclimate of local niches where mosquitoes were tested for survivorship. Independent sample t-test was performed to compare mean pupation rate, development time and microclimate differences across irrigated and other non-irrigated crop area. The analysis was performed using IMB SPSS Statistics 25, R 3.5.2 and Microsoft Excel 2016.

Results

Around 300 blood-engorged, *An. gambiae s.l.* were collected from indoor and outdoor (cow shelter) using mechanical mouth aspirators. Eight hundred first instar larvae hatched from the field-collected mosquitoes were used for the experiments in irrigated and non-irrigated areas, 400 each.

Developmental time and survivorship of *An. gambiae s.l.* larvae

The proportion of larvae that completed development from first instar larvae to pupae in irrigated area and non-irrigated area was 79.4 % (95%CI: 0.66 – 0.93) and 84.5% (95%CI: 0.77 – 0.92), respectively. Statistical analysis showed that the difference in pupation rate was not significant between irrigated and non-irrigated area (T-test = 2.22, P = 0.208) (Fig. 3). The mean larval-to-adult development time of *An. gambiae s.l.* larvae in irrigated area and non-irrigated area was 12.5 and 12, respectively. Similarly, the median larvae-to-pupae development time in irrigated was 12.5(95%CI: 10.2 – 14.8) days and at non-irrigated area 12(95%CI: 9.7 – 14.2) days (Table 1). Kaplan-Meier survival analysis showed no significant difference in larval survivorship between the two areas ($\chi 2 = 2.62$, $P < 0.106$) (Table 1).

Stage-specific survival and mortality analysis showed a slight increment in the mortality rate as the larvae develop to proceeding larval instars in both settings (Table 2).

Adult *An. gambiae s.l.* survivorship and fecundity

Survival analysis showed that female *An. gambiae s.l.* placed in the irrigated area survived significantly longer than those in the non-irrigated area ($\chi2 = 18.3$, df = 1, $P < 0.001$) (Fig. 4).

The estimated mean survival time of female *An. gambiae s.l.* in irrigated and non-irrigated area was 37.9 and 31.3 days, respectively (Table 3). Again, female mosquitoes showed the higher median survival period (41.0 days) in irrigated than non-irrigated area (31.0 days). A similar result was found that male *An. gambiae s.l.* survived longer period in irrigated than non-irrigated area ($\chi2 = 23.1$, df = 1, $P < 0.001$) with mean survival time of 31.8 and 24.2 days, respectively (Table 3). The median survival period for male mosquitoes was 33.0 days in irrigated area and 24.0 days in non-irrigated area (Table 1). Male *An. gambiae s.l.* survived shorter than females at both irrigated ($\chi2 = 14.9$, $P < 0.001$) and non-irrigated area ($\chi2 = 20.9$, $P < 0.001$) (Supplementary file 1).

Of 7,737 eggs laid by the female mosquitoes throughout the experiment period, 5,125 (66.2%) were from the mosquitoes placed in irrigated area and 2,612 (33.8%) were from mosquitoes in non-irrigated area. The study showed that fecundity of mosquitoes was 96.2% higher in irrigated area (80 eggs/day) than non-irrigated area (average 33 eggs/day). The mean eggs laid was (41±11.63 eggs/mosquito) and (21±5.61 eggs/mosquito) in irrigated area and non-irrigated area, respectively. Statistical analysis showed that the difference in fecundity was significant between irrigated and non-irrigated area (T-test = 2.83, P = 0.002) (Supplementary file 2).

Aquatic habitat microclimate during larval survivorship experiment

An independent sample t-test analysis on microclimate difference between the two study settings indicated that mean hourly water temperature (°C) in washbasins placed at non-irrigated area was by 1.1°C higher than washbasins in irrigated area (T-test = -2.85, P = 0.004). Similarly, mean light intensity (lum/ft2) in non-irrigated area (Mean = 497.4±982.2) was significantly higher than in irrigated area (Mean = 372.7± 664.8), (T-test = -2.47, P = 0.014) (Table 4 & Fig. 5). Mean maximum and minimum temperature and light intensity were also significantly higher in washbasins at non-irrigated area compared to irrigated area (Table 4).

Ambient microclimate during adult survivorship experiment

There was no significant difference in ambient hourly average, maximum and minimum temperature and relative humidity between irrigated area and non-irrigated area. However, mean light intensity between the two sites was different (P = 0.001) (Table 5 & Fig. 6).

Discussion

In this study, we investigated the effects of environmental modification on the development, survivorship and fecundity of malaria vector mosquitoes. We hypothesized that irrigated sugarcane plantation area enhances development, survivorship and fecundity compared to non-irrigated field crop area due to better microclimate and nutrients following environmental modification. However, the study showed no significant difference in development and survivorship of *An. gambiae s.l.* immatures between the two areas.

Variation in vegetation cover may affect the radiation flux and energy balance of the land surface and thus may modify the microclimate [25]. By the time experiment was conducted, sugarcane plantation was at its maturity stage, which is dense and leafy that partly could limit direct sunlight to reach to the washbasins, whereas in the surrounding crops field area were relatively less dense. The mean hourly water temperature in non-irrigated area increased by 1.1°C compared to irrigated area. This could partly explain the observed 5.1% more pupation rate in non-irrigated area compared to irrigated area. Studies reported elsewhere indicated that an increased temperature due to land use and land cover increased larval survival rate [10,26–30]. Tuno et al. [29] reported that the survivorship of *An. gambiae* larvae was reduced from 56 % in habitats fully exposed to sunlight to 1.5 % in habitats with forest canopy in western Kenya. Wang et al. [10] also reported pupation rate of *An. minimus*, malaria vector in china to be 52.5 %, 12.5 % and 3.8 % in the deforested, banana plantation and forested areas, respectively, which is far lower than our findings, 79.4 % and 84.5 % at irrigated and non-irrigated areas, respectively.

Nutrient availability may affect the survival, pupation rate and development time. The potential food source of anopheline larvae may include but not limited to bacteria, fungi, debris and organic matter. The abundance and structure of microbes such as algae and photosynthetic cyanobacteria in aquatic habitats may have changed in response to land use and land cover [31,32]. Organic matters and debris in the soil at different settings may not be the same, which could possibly vary with change in surrounding land use and land cover. Kebede et al. [33] reported that maize pollen provides nutrition for larval anopheline mosquitoes showing that the incidence of malaria was about 10 times higher in high maize-cultivation areas. In our case, the debris of sugarcane plantation and other field crops might not be the same but the result showed both areas are supporting mosquito development, which needs further investigation of a soil's biological and chemical composition in relation to mosquito immatures development.

The higher pupation rate and longer survivorship of *An. gambiae s.l.* immatures generally could increase vectorial capacity to enhance malaria transmission. Based on these findings alone, we cannot conclude that the irrigated area is encountering less or equal malaria risk compared to surrounding environs. Recently, in the study conducted from the same area, significantly more diverse breeding sites and larvae abundance in irrigated sugarcane plantation area than its surrounding has been reported [23]. Thus, more diversified breeding sites with 79.4 % pupation rate could certainly outweigh the malaria burden over surrounding environs with less habitat diversity and relatively the same pupation rate.

Adult *An. gambiae s.l* placed in the irrigated area survived longer than non-irrigated area. Adult female mosquitoes survived longer than male in both settings. Our findings of mosquito longevity was in line with previous studies elsewhere. For instance, Okech et al. [34], reported mean survival of 33 days for *An. gambiae s.l* in western Kenya, which is 6 days shorter than our finding. Gary and Forster [35], found that *An. gambiae s.l.* mosquitoes had a median survival time of 29 days under insectary conditions, but in our study, the median survival time for female *An. gambiae s.l.* was 41 and 31 days at irrigated area and non-irrigated area, respectively. The longer survival of mosquito at the irrigated area indicates that *An. gambiae s.l.* is well adapted to the environmental conditions. Enhanced survival of malaria vector is among the determinants of increased mosquito vectorial capacity [36]. A long-lived adult female mosquito increases opportunities to encounter an infected human host, the extrinsic incubation period of malaria parasites and reach the salivary glands after an infective blood meal, and transmission of parasites in later blood meals to uninfected hosts [1,3,37]. Thus, it has an implication on malaria transmission at the locality.

The experiment set-up at both study settings made the same and human blood and sugar were provided in a similar way. Thus, the only difference was the environment where the experiments were situated, being irrigated area and non-irrigated area. There was no significant difference in mean, maximum and minimum hourly ambient temperature; and relative humidity between two environments. Previous studies indicated that *An. arabiensis,* a primary vector in Ethiopia, generally prefers areas with low humidity and high temperature [38]. A similar study also demonstrated that reduced humidity and increased temperatures following deforestation creates a more suitable environment for adult *An. arabiensis* to survive longer [26]. Therefore, in our study setting the determinants involving in supporting better survival of adult *An. gambiae s.l.* at irrigated area warrants further investigation.

The average daily fecundity of *An. gambiae s.l.* mosquitoes in irrigated area was 96.2 % higher compared to non-irrigated area. Increased survival together with enhanced fecundity of malaria vector in irrigated area suggests that the longevity and biotic potential of *An. gambiae s.l.* in the area is very high, favoring increased population density and thus the species could contribute much to malaria transmission. Better survival and fecundity in the irrigated area in our study is in agreement with the study conducted in Ethiopia at the laboratory level demonstrated that gravid *An. arabiensis* females attracted to sugarcane pollen volatiles [39].

This study had several limitations. The experiment was done in one time of the maturity stage of irrigated sugarcane area. The microclimate conditions in irrigated area during seedling/ germinating stage, tillering stage, grand growth stage & maturity stage [40] could not be the same which in turn influence the mosquito survivorship. The information on chemical and nutrient's composition of a soil used as a substrate was not included in the study. Moreover, the experiments were conducted under controlled condition of all potential biological factors that may influence mosquito survival like predators and competitors, which might possibly leads to overestimated survival time than actual.

Conclusion

Irrigated sugarcane plantation significantly enhances the survivorship and fecundity of adult *An. gambiae s.l.,* the major malaria vector in Ethiopia. The study results on survivorship parameters of malaria vector mosquitoes under a variety of environmental conditions is helpful to model impact of environmental modification on vector population dynamics and help devise tailor-made vector control strategies. Moreover, longer survivorship of adult mosquito in irrigated area calls for the need for larval management to reduce the vector population and subsequent malaria transmission

Declarations

Authors' contributions

DH conceived the study; conducted experiment; performed data analysis, interpreted data and drafted a manuscript. DB involved in data analysis. AD and AT involved in data acquisition. DY, SK and GY critically reviewed the manuscript for important intellectual content. All authors read and approved the final manuscript.

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Competing interests

The authors declare that they have no competing interests.

Availability of data and materials

The datasets used and/or analysed during the current study are available from the corresponding author on reasonable request.

Consent for publication

Not applicable.

Ethics approval and consent to participate

Ethical clearance was obtained from the Institutional Review Board (IRB) of Institute of Health, Jimma University, National Ethics Review Committee (NERC) of Ethiopia and University of California, Irvine, USA. The study did not involve any endangered or protected species.

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Tables

Table 1 Means and medians of survival time for immature *An. gambiae s.l.* in irrigated and non-irrigated areas, Southwest Ethiopia, 2019.

Site	Mean with 95%CI	Median with 95%CI	Overall comparisons		
Irrigated area	12.5 (10.3 - 14.4)	12.5 (10.2 – 14.8)	χ2	df	p-value
Non-irrigated area	12.1 (11.6 - 13.9)	12.0 (9.7 – 14.2)	2.62	1	0.106

Table 2 Stage-specific survivorship and mortality rate of immature *An. gambiae s.l.* in irrigated and non-irrigated areas, Southwest Ethiopia, 2019.

Stage	Irrigated area			Non-irrigated area		
	Development time (day)	Cumulative survival rate	Stage mortality rate	Development time (day)	Cumulative survival rate	Stage mortality rate
1st instar	2.3	0.98	0.02	2.1	0.98	0.03
2nd instar	2.5	0.96	0.03	2.4	0.95	0.03
3rd instar	4.6	0.92	0.04	3.5	0.89	0.06
4th instar	5.1	0.79	0.19	4.5	0.84	0.09

Table 3 Means and medians of survival time for adult *An. gambiae s.l.* in irrigated and non-irrigated areas, Southwest Ethiopia, 2019.

Site	Female *anopheles* gambiae		Male *anopheles* gambiae	
	Mean with 95%CI	Median with 95%CI	Mean with 95%CI	Median with 95%CI
Irrigated area	37.9 (34.8 – 41.5)	41.0 (35.9 – 46.1)	31.8 (28.9 – 34.7)	33.0 (28.3 – 37.7)
Non-irrigated area	31.3 (28.5 – 34.1)	31.0 (27.9 – 34.1)	24.2 (21.8 – 26.6)	24.0 (20.3 – 27.6)

Table 4 Mean hourly temperature and light intensity in washbasins in irrigated and non-irrigated areas, Southwest Ethiopia, 2019.

Microclimate	Irrigated area (M±SE)	Non-irrigated area (M±SE)	t	df	P
Mean temperature (°C)	23.3±5.7	24.4±6.3	-2.85	1068	0.004
Mean maximum temperature (°C)	24.4±6.5	25.4±7.2	-2.53	1068	0.012
Mean minimum temperature (°C)	22.5±5.1	23.4±5.4	-2.83	1068	0.005
Mean light intensity (lum/ft^2)	372.7± 664.8	497.4±982.2	-2.47	1068	0.014
Mean maximum light intensity (lum/ft^2)	713.0±1311.7	931.0±1698.0	-2.28	1068	0.018
Mean minimum light intensity (lum/ft^2)	174.4±311.9	229.1±495.5	-2.21	1068	0.027

M±SE, Mean ± Standard Error

Table 5 Hourly microclimate condition of mosquito niches in irrigated and non-irrigated areas, Southwest Ethiopia, 2019.

Microclimate	Irrigated area (M±SE)	Non-irrigated area (M±SE)	t	df	P
Mean temperature (°C)	21.56±4.80	21.60±4.81	-0.26	3176	0.790
Mean maximum temperature (°C)	22.22±5.09	22.24±5.10	-0.09	3176	0.927
Mean minimum temperature (°C)	20.90±4.56	20.92±4.56	-0.12	3176	0.904
Mean relative humidity (%)	82.65±15.73	82.30±14.58	-0.63	3176	0.522
Mean maximum relative humidity (%)	86.11±13.77	86.55±11.80	-0.92	3176	0.339
Mean minimum relative humidity (%)	78.95±17.78	78.12±17.23	1.31	3176	0.187
Mean light intensity (lum/ft^2)	324.3±517.5	709.0±1242.3	-11.7	2952	0.001
Mean maximum light intensity (lum/ft^2)	571.7±982.5	1106.8±1834	-10.3	2952	0.001
Mean minimum light intensity (lum/ft^2)	180.1±267.5	366.6±663.4	-10.7	2952	0.001

M±SE, Mean ± Standard Error

Figures

Figure 1

Insect-proof BugDorm tent with washbasins inside

Figure 2

Roof structure from which cages with adult An. gambiae s.l. mosquitoes suspended

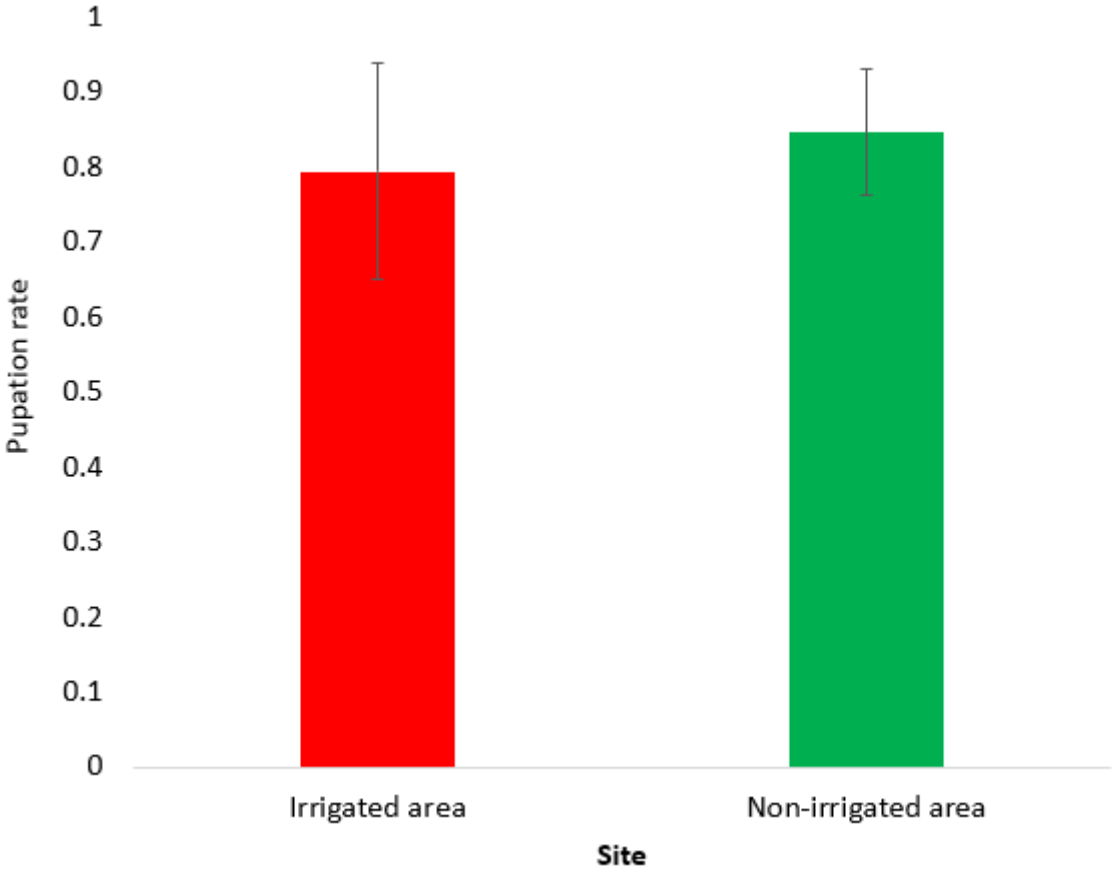

Figure 3

Pupation rate of An. gambiae s.l. larvae in irrigated and non-irrigated areas, Southwest Ethiopia, 2019.

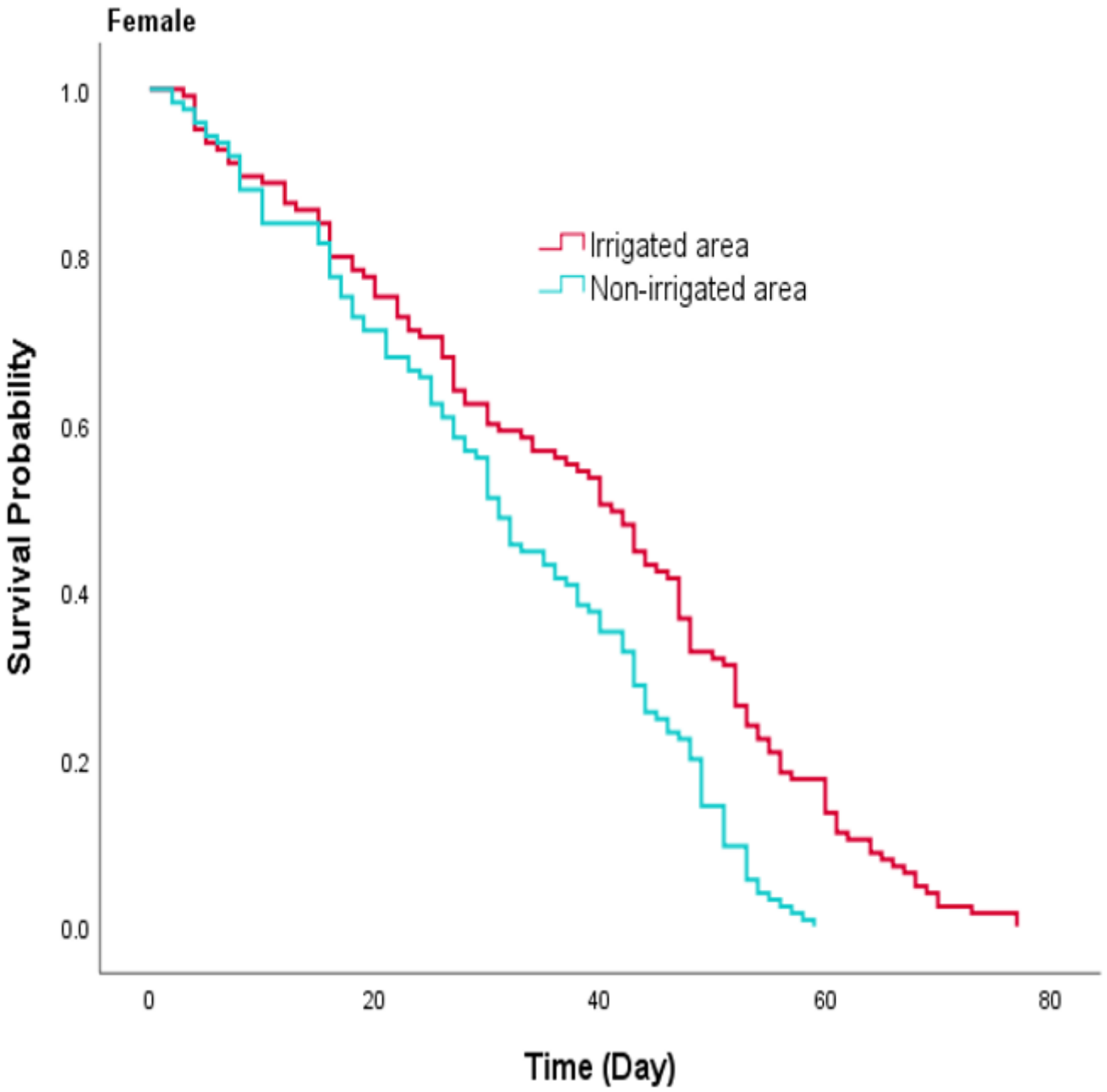

Figure 4

Survivorship of adult An. gambiae s.l. in irrigated and non-irrigated areas, Southwest Ethiopia, 2019.

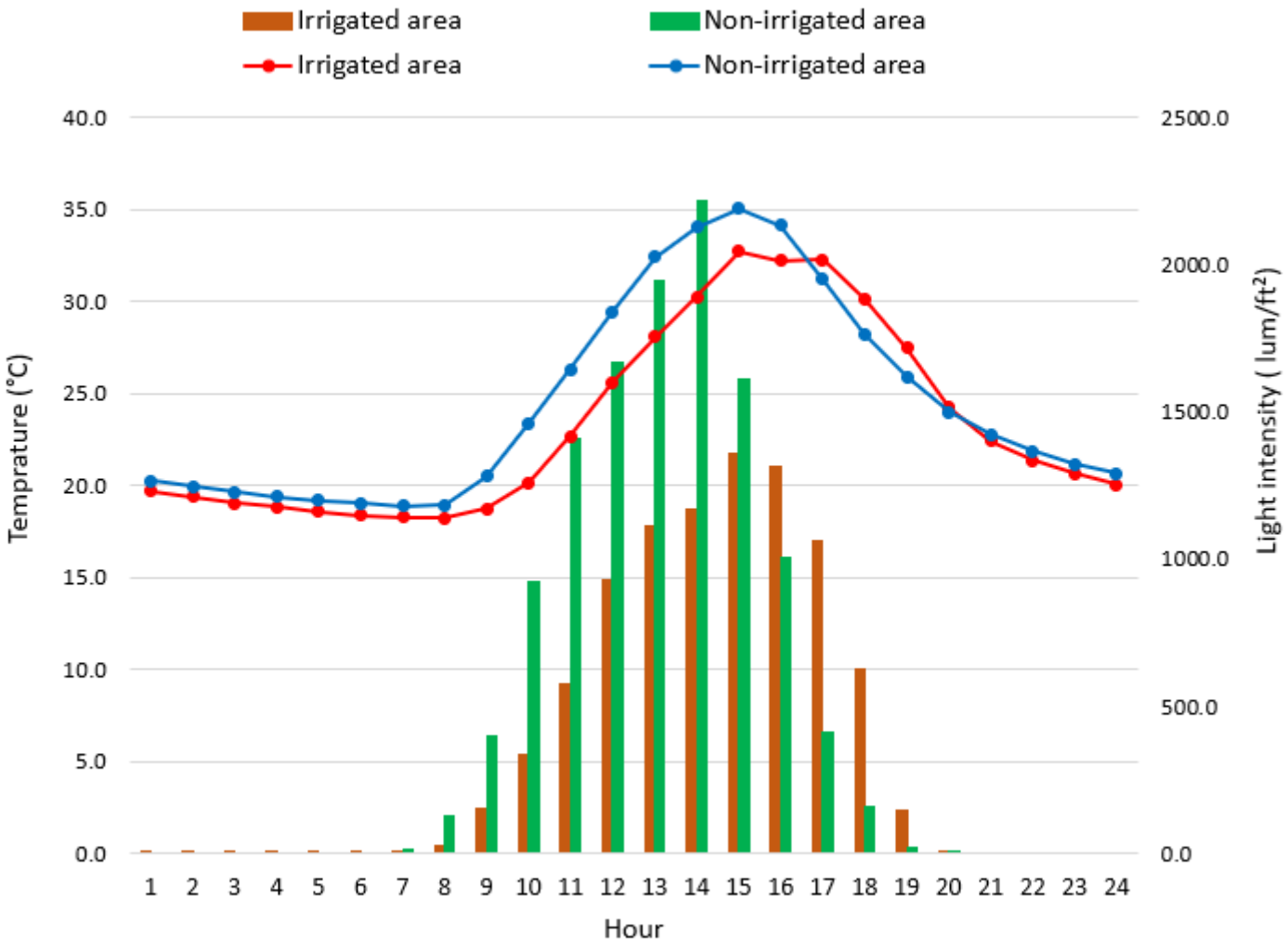

Figure 5

Mean hourly temperature and light intensity 24-hour daily cycle in washbasins in irrigated and non-irrigated areas, Southwest Ethiopia, 2019.

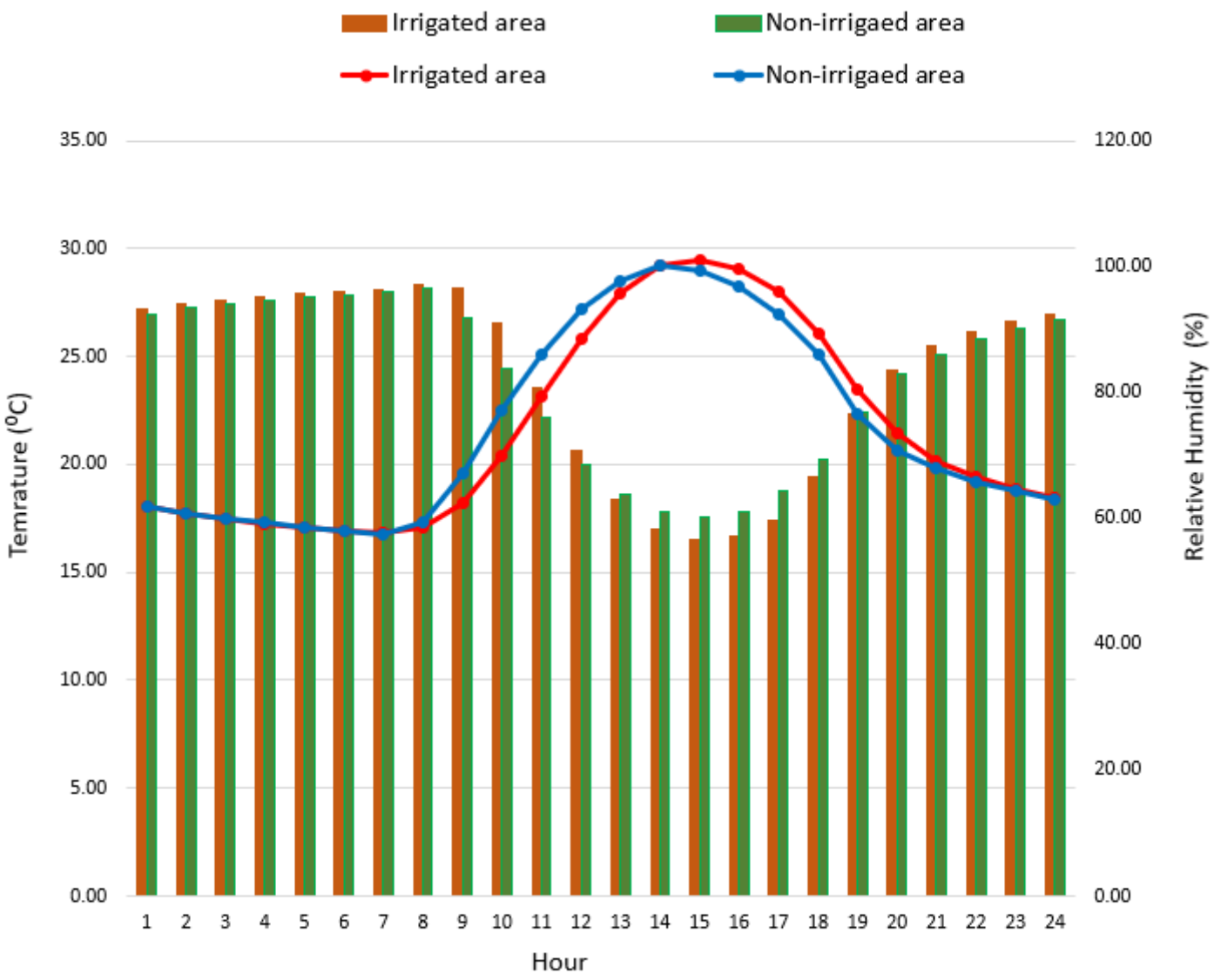

Figure 6

Mean hourly ambient temperature and relative humidity 24-hour daily cycle in irrigated and non-irrigated areas, Southwest Ethiopia, 2019.

Supplementary Files

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- Graphicalabstract.jpg
- Supplementaryfile1.docx
- Supplementaryfile2.docx