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A low-cost mesocosm for the study of behaviour and reproductive potential of Afrotropical mosquito (Diptera: Culicidae) vectors of malaria

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Abstract

A large-scale mesocosm was constructed and tested for its effectiveness for experiments on behaviour, reproduction, and adult survivorship of the Afrotropical malaria vector *Anopheles gambiae s.s.* Giles (Diptera: Culicidae) in temperate climates. The large space (82.69 m^3) allowed for semi-natural experiments that increased demand on a mosquito's energetic reserves in an environment of widely distributed resources. A one-piece prefabricated enclosure, made with white netting and vinyl, prevented the ingress of predators and the egress of mosquitoes. Daylight and white materials prompted the mosquitoes to seclude themselves in restricted daytime resting sites and allowed easy collection of dead bodies so that daily mortality could be assessed accurately, using a method that accounts for a proportion of bodies being lost. Here, daily, age-dependent mortality rates of males and females were estimated using Bayesian Markov Chain Monte Carlo simulation. In overnight experiments, mosquitoes successfully located plants and took sugar meals. A 3-week survival trial with a single-cohort demonstrated successful mating, blood feeding, oviposition, and long life. The relatively low cost of the mesocosm and the performance of the mosquitoes in it make it a viable option for any behavioural or ecological study of tropical mosquitoes where space and seasonal cold are constraining factors.

Keywords

Anopheles gambiae; cohort survival; large mesocosm; mosquito behaviour; semi-natural enclosure; sugar feeding

In recent years, the use of semi-field systems has gained recognition and popularity among vector ecologists, in part driven by a need to understand aspects of gene flow and relative fitness of genetically- or biologically-modified mosquitoes (Knols *et al.*, 2002; Ferguson *et*

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al., 2008; Fachinelli *et al.*, 2011; Ritchie *et al.*, 2011). Such evaluations depend on accurate and meaningful measures of life-history parameters, such as age-specific survivorship, fecundity, and mating behaviour. Several studies have demonstrated that the decreased flight activity associated with small laboratory cages can misrepresent natural energetic intake and expenditure (Stone *et al.*, 2011), resulting in artificially high insemination and survivorship rates (Gary *et al.*, 2009; Ponlawat & Harrington, 2009; Stone *et al.*, 2009a). It is also paramount that the design allows for an accurate assessment of the standing population of both males and females, but obtaining reliable estimates of population sizes and daily mortality rates represents a methodological issue for mesocosm or semi-field studies. Direct counts of surviving females resting along the walls or in resting sites are imprecise unless every individual is located, and distinguishing between males and females within their resting sites can be difficult. Successive random subsamples of the population (Stone *et al.*, 2009b; Ritchie *et al*., 2011) can also be used, but drawbacks of this method are inherent sampling error and either a consequently diminished population or the mosquito stress created by removal-replacement sampling. Further, this method and indirect measures, such as egg production or counts of biting females, become biased if the treatment of interest (e.g., environmental conditions) influences the physiology or behaviour of the mosquito or if mortality is not constant, as has been found for field and laboratory populations (Clements & Paterson, 1981; Styer *et al*., 2007).

Here, a cost-effective, easily erected enclosure that addresses the issues of flight space, population estimation, and exclusion of unwanted predators and nectar robbers is described. Data on both sexes of *Anopheles gambiae* from an experiment that tested their ability to locate and feed on plants and from an experiment on the survival, biting, and reproductive behaviour over an extended period of time when they had access to plants and a human host are presented.

From plans, MegaView Science Co., Ltd. (Taichung, Taiwan), a pair of one-piece customized enclosures that required only construction of supporting frames before installation were developed. The two mesocosms were erected within two adjacent rooms of The Ohio State University Biological Sciences Greenhouse (39° 59’ 47” N 83° 1’ 3” W), each with a 44.5 m^2 concrete floor, a 1-m-high concrete block wall on all four sides, with glass panels above them and on the ceiling. The pair of mesocosms later would allow simultaneous comparison of different experimental treatments (see Stone *et al.*, 2012). Dimensions of a single mesocosm were 5.66 × 4.87 × 3.00 m (L × W × H) for a total of 82.69 m^3 (Fig. 1). The mesocosm sides, ceiling, and sleeves were made of white polyester netting (42 × 12 mesh per sq. cm, 470 μm mesh aperture), whereas the floor material was white vinyl. To protect the vinyl floor, a 4.57 × 6.10 m white tarp (Tarpaflex US, Naples, FL) was placed inside the mesocosm. The mesocosm was suspended on a framework of PVC connectors and pipes cut from 40 pieces of 3.81 × 304.8 cm (1.5 in × 10 ft) PVC pipe, purchased from a local hardware store, and also specialty 3-way and 4-way connectors (AFC Greenhouses, Buffalo Junction, VA). Pipes and connectors were secured with small screws. Pairs of 0.46-m-long ties, located every 0.4 – 0.5 m along all horizontal and vertical edges and across the ceiling, allowed the mesocosm to be tied to the PVC framework and the greenhouse ceiling joists. Nine cylindrical sleeves (0.45 m diameter and 0.5 m length)

made from the polyester netting were located in the ceiling to allow nine 400-W metal-halide wide-spectrum grow lights to be suspended inside the mesocosm. The cylindrical sleeves were then wrapped around chains supporting the lights and held in place with plastic zip ties. Located on the floor were two zippered drains, covered by vinyl flaps. Each drain was 0.18 m^2, with netting beneath the flap to allow water to drain through. The position of the drains on the vinyl floor corresponded to drains in the concrete greenhouse floor. Access to the mesocosm was through a large D-shaped zippered door (1.8 m high and 1.2 m wide) made from clear vinyl, positioned in the middle of one of the short walls. Outside of the door was a small 1 × 2 × 2 m antechamber to prevent mosquito escape. The doors to the antechamber could be partially unzipped to allow a person to enter or exit while minimizing the chance of mosquitoes escaping. When fully unzipped, they allowed import of large plants. On the vertical wall, left of the antechamber, were two sleeves, one for the humidifier exhaust pipe, the other for the humidistat controls (see below).

Inside of the mesocosm were four resting sites and two oviposition sites. Clay pots, placed on their sides, served as resting sites. Each measured 32 × 37 × 22 cm (height × opening diameter × base diameter). The pot opening was covered with a circular plywood insert with a 13-cm hole in the middle, which allowed the mosquitoes to enter and exit the pots. These pots were soaked in water before the start of each experiment and intermittently moistened to increase the relative humidity of these harbourages. Oviposition sites were 31-litre clear plastic storage bins (58 × 38 × 17 cm), containing 5 litres of aged tap water and seated on dark fabric to contrast with the white floor. (See Stone *et al*., 2012 for account of oviposition and method used to estimate egg numbers). These bins were not used for development of hatched larvae in the overnight and single-cohort tests, though they could serve that purpose, for example in studies of overlapping generations to investigate the spread of refractory genotypes. Mosquitoes used for experiments were insectary-reared in pans at 100 larvae per pan containing 450 ml of water and maintained on a diet of powdered Tetramin fish flakes, as previously described (Stone *et al*., 2009a).

Temperature was controlled within broad limits by the greenhouse heating and cooling systems. Wall-mounted steam radiators heated the room while cooler temperatures were maintained by louvered roof vents and a 1/3 horsepower exhaust fan that pulled air through an evaporative cooling system spanning the opposite wall of the greenhouse. Both systems were controlled by a thermostat within a sensor module suspended in the middle of the mesocosm about 1 m from the floor. Humidity was maintained with an Ocean Mist® MH3 industrial ultrasonic humidifier (Mico Inc., El Monte, CA), which was controlled by a humidistat located adjacent to the sensor module. A solenoid-actuated gate was fitted to the end of the humidifier exit pipe to prevent mosquitoes from flying into the humidifier when it was not running. The grow lights were programmed to turn on 30 min after sunrise and turn off 30 min prior to sunset, but within that photophase they were on only when outdoor lighting dropped below 15,000 lux. The delayed onset and early termination of lighting allowed for more natural, less abrupt morning and evening crepuscular periods.

The north and south walls of the greenhouse room were covered with reflective insulation (FarmTek, Dyersville, IA) and the hallway-facing wall (east) with 6-mil black plastic sheeting to block artificial light from other sources after sunset. The outer sunset-facing wall

(west) was covered with black polypropylene commercial shade cloth (60% shade) (Hummert International, Earth City, MO). It was also suspended above the mesocosm. The shade cloth shielded the mesocosm from direct sunlight, helping to maintain temperatures within the specified range and minimizing damaging effects of ultraviolet light on the netting. At mid-day on a sunny day (29 May 2012), with the grow lights turned off, the light intensity at floor level of an empty greenhouse room, lacking shade cloth and mesocosm netting, was 79,600 lux, as measured by light meter (LX-1010BS). Inside the fully constituted mesocosm the light intensity was 14,830 lux, illustrating the need for the grow lights to provide more light.

To minimize extraneous artificial light, a long and wide strip of reflective insulation was installed on the window at each corner of the western wall. An artificial horizon (inspired by Marchand, 1985) was created along the exposed glass windows by covering their lower portions, up to about 1.5 m, with an opaque black cloth. Male swarms and pair formation were observed at dusk along this wall, particularly at the dark-light horizon and near the corners where the window met the insulation. This is reminiscent of the description of certain *An. funestus* swarms in Mozambique occurring in nature where gaps in the vegetation provide an illuminated sky (Charlwood *et al*., 2003) or of *An. gambiae* s.l. in Tanzania where the use of horizon markers was implicated (Marchand, 1984), but differs from recent accounts of the M and S forms of *An. gambiae s.s*., reported to occur above swarm markers (Sawadogo *et al*., 2014). To find out whether this is a modification of the swarming habit of this species due to the conditions in the mesocosm, a reflection of a difference between East and West African populations, or a consequence of colonization and inbreeding, will require a detailed study using F1 generations of wild-type mosquitoes.

The total cost of a single mesocosm setup was 3,123.00 US dollars. This included the prefabricated mesocosm, PVC framework, humidifier and its accompanying parts, tarps, resting sites, and oviposition sites. Having available space in a greenhouse kept costs down, because it provided a suitable structure to house the mesocosm and control temperature.

Foraging for nectar by males was observed in the mesocosm. Four replicates of overnight assays were performed to quantify the extent of sugar feeding in the mesocosm when mosquitoes had access to various potted plants (five *Tithonia diversifolia* and one *Senna didymobotrya)*, but no blood source. Mosquitoes (approximately 200 males and females were used for each replicate) were introduced into the mesocosm the afternoon after they emerged, and collected by backpack and mouth aspirators 1–2 h after sunrise of the following morning. A random sample of 30 males and 30 females of those collected per replicate (n = 240) were kept in a −40°C freezer until assayed with a cold-anthrone test for the presence and estimation of the amount of ingested fructose (Haramis & Foster, 1983). The results are shown in Figs. 2A, B. and were analyzed using JMP 9 statistical software (SAS, Raleigh, NC). Fructose positivity was affected by both sex (GLM: binomial logit: $\chi^2 = 40.3$; df = 1; $P < 0.0001$) and replicate night (GLM: binomial logit: $\chi^2 = 24.6$; df = 1; $P < 0.0001$). The rate of sucrose positivity of all replicates combined, was 74.7% for males and 36.6% for females. A comparison of the amounts of fructose detected a significant interaction between sex and replicate (Kruskal-Wallis = 15.64, df = 5, $P = 0.008$). Differences in fructose positivity and fructose amount among replicates may have been

caused by changes in plant health. Being maintained in a horticultural greenhouse, occasional infestation of our plants, particularly castorbean, *Ricinus communis*, and *S. didymobotrya*, with two-spotted spider mite, *Tetranycheus urticae*, was unavoidable and to our knowledge the main driver of detrimental changes in plant health. Some plants, such as castorbean and lima bean (*Phaseolus lunatus*) are known to increase extrafloral nectar (EFN) production in response to herbivory, presumably as an indirect defense intended to attract and arrest predators and parasitoids (Wäckers *et al.*, 2001; Choh *et al*., 2006). This, or a reduction in nectar production (as a result of a deteriorating state due to infestation) could affect the sugar-feeding behaviour and survivorship of mosquitoes in mesocosm experiments. To account for this potential source of variability, in future experiments it would be useful to measure EFN production using capillary tubes and treat this as a covariate (as one could do with temperature and relative humidity). The implications of such variability in nectar production for sugar feeding in nature are that static preferences for certain plant species may be too narrow, and instead mosquitoes may benefit from being able to locate or return to highly productive individual plants.

A survival experiment was conducted, in which 833 *An. gambiae s.s.* adults emerged from pupae in the mesocosm, where the adults had access to eight nectariferous plant species identified as possible hosts for this mosquito (Manda *et al.*, 2007), four resting sites, a human blood source (B.E.) (1h nightly) (IRB permit 2004H0193, IBC permit 2005R0020), and two oviposition sites. In an earlier study, the number of bites per female was measured by providing access to a human host at dawn and simply counting the bites received (Stone *et al*., 2012). In this experiment and a follow-up study (Ebrahimi *et al*., in prep.), a blood host was present for an hour around midnight, and counts were made based on infrared video recordings of the exposed lower legs of the volunteer. Daily mortality could be assessed because dead bodies were noticeable in contrast to the white floor and could be easily collected. Resting sites as well as plant leaves and plant-holding pots were checked for dead bodies. Average daily egg production for surviving females was 33 (range 13–51). Any bites occurring during these times may have gone unnoticed, but female activity in general was low during the daytime. At the termination of the experiment, all surviving females were frozen and found to be inseminated upon dissection and microscopic inspection of the spermatheca for the presence of sperm. A proportion of mosquitoes were lost throughout the experiment, as there was a discrepancy between the total number of mosquitoes released and recovered alive or dead. Previously, it was assumed that a constant proportion of bodies was overlooked and that the counts could therefore be adjusted accordingly, leading to point estimates of the number of survivors per day (Stone *et al*., 2012). Another source of uncertainty was due to the release of pupae of which the sex was not determined, rather than using methods requiring a higher degree of handling and interfering with early life sequences of adults, such as aspirating and counting newly emerged adults from a cage or allowing pupae to emerge into individual stoppered vials before releasing them. A deviation from a 50% sex ratio is possible because males tend to emerge sooner than females, although this can be mitigated by staggering the hatching of eggs.

Here, the two sources of uncertainty in the data were revisted and statistical properties of mortality estimates were obtained, rather than point estimates only. To do so, it was assumed that the initial number of males and females followed a binomial distribution, $A_{0,s} \sim Bin(\alpha, N_p)$, where α is the assumed sex ratio of 0.5, N_p the number of eclosed pupae, and the subscript s denotes sex. For each day, it was assumed that a proportion $\mu_{i,s}$ of the population suffers mortality, and that a constant proportion λ_s of those dead mosquitoes will be recovered the following morning. Therefore, the assumption that lost mosquitoes disappeared after dying was made (i.e., they were overlooked or carried off by ants) and that the cage prevented dispersal of live mosquitoes. The number of dead mosquitoes per day was then given by: $L_{i,s} \sim Bin(\mu_{i,s}, A_{i-1,s})$, the number of recovered (dead) mosquitoes per day follows $D_{i,s} \sim Bin(\lambda, L_{i,s})$, and $A_{i,s} = A_{i-1,s} - L_{i,s}$. The values for $\mu_{i,s}$ and $\lambda_{i,s}$ were estimated using uninformative, uniform prior distributions between 0 and 1 using a Bayesian approach. Specifically, the parameter values were estimated using the Bayesian Markov Chain Monte Carlo simulation program JAGS, run in R via the package R2jags. The mean and 95% confidence intervals for daily mortality estimates for females and males are depicted in Figure 3.

The estimated daily mortality rates for males and females 1) were very low compared to field estimates derived from mark-release-recapture studies, for instance, in a study in Tanzania the daily mortality rate was estimated at 0.22 per female (Takken *et al*., 1998), the difference owing, in part, to the lack of extrinsic mortality factors such as predation or defensive behaviour of blood hosts; 2) were very similar for males and females, suggesting that sufficient sugar was available, particularly to sustain males, and conditions in the resting pots were conducive to survival; 3) increased with age. This speaks to the usefulness of such temperate-setting mesocosms for the study of tropical mosquito demography and the influence of environmental conditions or vector control interventions thereupon. The Bayesian modelling approach used was highly flexible and could be particularly useful for semi-field experiments when determining the contribution of intrinsic and extrinsic factors to mortality is of interest.

The average recorded temperature and relative humidity inside the mesocosm were 25.2°C (range: 20.7—33.7°C) and 71% (range: 50—81%), respectively, which is comparable to the temperature range described for a semi-field system in Tanzania (Ferguson *et al*., 2008). The average temperature and humidity in one resting pot were 22.7°C (range: 19.2—26.7°C) and 86% (range: 67—100%), respectively.

While maintaining stable overlapping generations within this enclosure was not attempted, it was possible to show that the equatorial malaria vector *An. gambiae* survived and engaged in its normal behaviours related to mating, foraging for blood and sugar, and resting (Takken & Knols, 1999). The enclosure described here is easy to set up and maintain, inexpensive, and sufficiently spacious to allow for a variety of behavioural and population-level investigations. The approach to approximate semi-natural situations in temperate zones is likely to be useful for studies of other mosquito species, and it will be particularly relevant when natural energetic demands and the implications thereof on survival, reproductive potential, and behaviour could significantly influence the outcome of the study.

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References Cited

Charlwood JD, Thompson R, Madsen H. Observations on the swarming and mating behaviour of *Anopheles funestus* from southern Mozambique. Malaria Journal. 2003; 2:2. [PubMed: 12636875]

Choh Y, Kugimiya S, Takabayashi J. Induced production of extrafloral nectar in intact lima bean plants in response to volatiles from spider mite-infested conspecific plants as a possible indirect defense against spider mites. Oecologia. 2006; 147:455–460. [PubMed: 16341892]

Clements AN, Paterson GD. The analysis of mortality and survival rates in wild populations of mosquitoes. Journal of Applied Ecology. 1981; 18:373–399.

Facchinelli L, Valerio L, Bond JG, Wise de Valdez MR, Harrington LC, Ramsey JM, et al. Development of a semi-field system for contained field trials with *Aedes aegypti* in southern Mexico. American Journal of Tropical Medicine and Hygiene. 2011; 85:248–256. [PubMed: 21813843]

Ferguson HM, Ng'habi KR, Walder T, Kandungula D, Moore SJ, Lyimo I, et al. Establishment of a large semi-field system for experimental study of African malaria vector ecology and control in Tanzania. Malaria Journal. 2008; 7:158. [PubMed: 18715508]

Gary RE, Cannon JW III, Foster WA. Effect of sugar on male *Anopheles gambiae* Giles (Diptera: Culicidae) mating performance, as modified by temperature, space, and body size. Parasites and Vectors. 2009; 2:19. [PubMed: 19386114]

Haramis LD, Foster WA. Visual quantification of sugar in mosquitoes using anthrone reagent. Mosquito News. 1983; 43:362–364.

Knols BGJ, Njiru BN, Mathenge EM, Mukabana WR, Beier JC, Killeen GF. MalariaSphere: A greenhouse-enclosed simulation of a natural *Anopheles gambiae* (Diptera: Culicidae) ecosystem in western Kenya. Malaria Journal. 2002; 1:19. [PubMed: 12537599]

Manda H, Gouagna LC, Nyandat E, Kabiru EW, Jackson RR, Foster WA, et al. Discriminative feeding behaviour of *Anopheles gambiae s.s.* on endemic plants in western Kenya. Medical and Veterinary Entomology. 2007; 21:103–111. [PubMed: 17373953]

Marchand RP. Field observations on swarming and mating in *Anopheles gambiae* mosquitoes in Tanzania. Netherlands Journal Zoology. 1984; 34:367–387.

Marchand RP. A new cage for observing mating behavior of wild *Anopheles gambiae* in the laboratory. Journal of the American Mosquito Control Association. 1985; 1:234–236. [PubMed: 3880236]

Ponlawat A, Harrington L. Factors associated with male mating success of the dengue vector mosquito, *Aedes aegypti* . American Journal of Tropical Medicine and Hygiene. 2009; 80:395–400. [PubMed: 19270288]

Ritchie SA, Johnson PH, Freeman AJ, Odell RG, Graham N, De Jong PA, et al. A secure semi-field system for the study of *Aedes aegypti* . PLoS Neglected Tropical Diseases. 2011; 5:e988. [PubMed: 21445333]

Sawadogo PS, Namountougou M, Toé KH, Rouamba J, Maïga H, Ouédraogo KR, et al. Swarming behaviour in natural populations of *Anopheles gambiae* and *An. coluzzii*: Review of 4 years survey in rural areas of sympatry, Burkina Faso (West Africa). Acta tropica. 2014; 130:24–34.

Stone CM, Taylor RM, Roitberg BD, Foster WA. Sugar deprivation reduces insemination of *Anopheles gambiae* (Diptera: Culicidae), despite daily recruitment of adults, and predicts decline in model populations. Journal of Medical Entomology. 2009a; 46:1327–1337. [PubMed: 19960677]

Stone CM, Taylor RM, Foster WA. An effective indoor mesocosm for studying populations of *Anopheles gambiae* in temperate climates. Journal of the American Mosquito Control Association. 2009b; 25:514–516. [PubMed: 20099601]

Stone CM, Hamilton IM, Foster WA. A survival and reproduction trade-off is resolved in accordance with resource availability by virgin female mosquitoes. Animal Behaviour. 2011; 81:756–774.

Stone CM, Jackson BT, Foster WA. Effects of plant-community composition on the vectorial capacity and fitness of the malaria mosquito *Anopheles gambiae* . American Journal of Tropical Medicine and Hygiene. 2012; 87:727–736. [PubMed: 22927493]

Styer LM, Carey JR, Wang J, Scott TW. Mosquitoes do senesce: departure from the paradigm of constant mortality. American Journal of Tropical Medicine & Hygiene. 2007; 76:111–117. [PubMed: 17255238]

Takken W, Charlwood JD, Billingsley PF, Gort G. Dispersal and survival of *Anopheles funestus* and *A. gambiae s.l.* (Diptera: Culicidae) during the rainy season in southeast Tanzania. Bulletin of Entomological Research. 1998; 88:561–566.

Takken W, Knols BGJ. Odor-mediated behavior of Afrotropical malaria mosquitoes. Annual Review of Entomology. 1999; 44:131–157.

Wäckers FL, Zuber D, Wunderlin R, Keller F. The effect of herbivory on temporal and spatial dynamics of foliar nectar production in cotton and castor". Annals of Botany. 2001; 87:365–370.

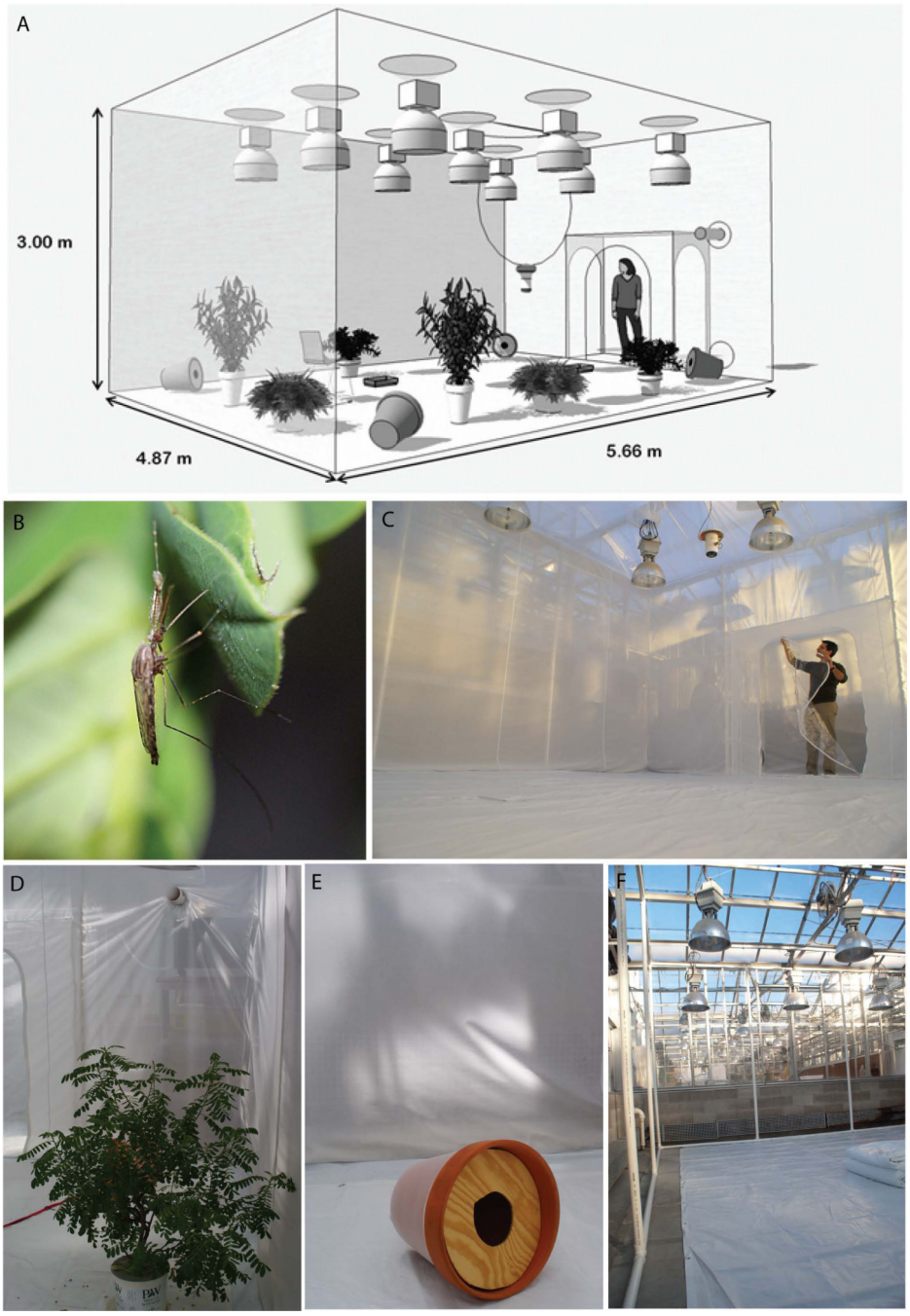

Fig. 1.

(A) Schematic drawing of a mesocosm as it appeared during experimentation. (B) A male An gambiae s.s. resting on a Senna didymobotrya. (C) Metal-halide grow lights and the mesocosm before use. (D) Humidifier exhaust pipe and S. didymobotrya. (E) Resting site. (F) PVC structure and greenhouse room prior to set up of the mesocosm.

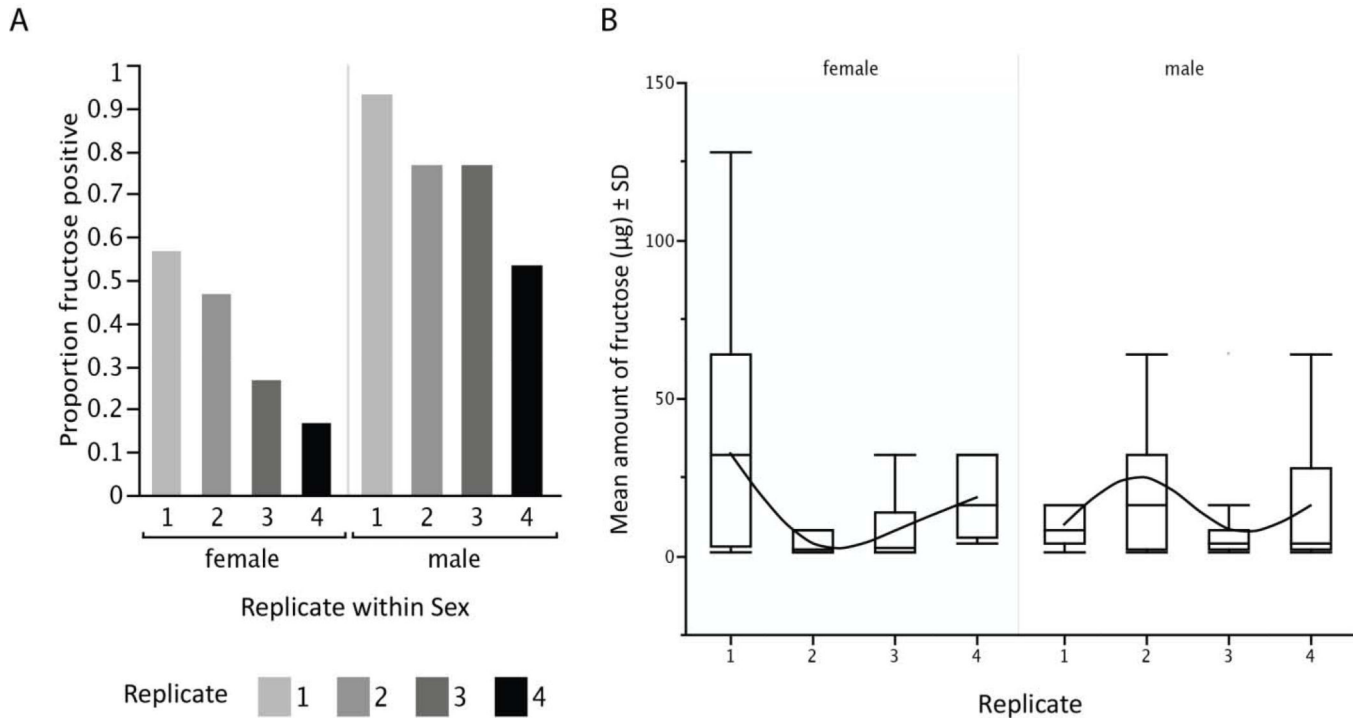

Fig. 2.

(A) Proportion of An. gambiae s.s. males and females positive for fructose after being held in a mesocosm overnight where they had access to six plants. (B) Mean amount of fructose (µg) ± SD for both males and females.

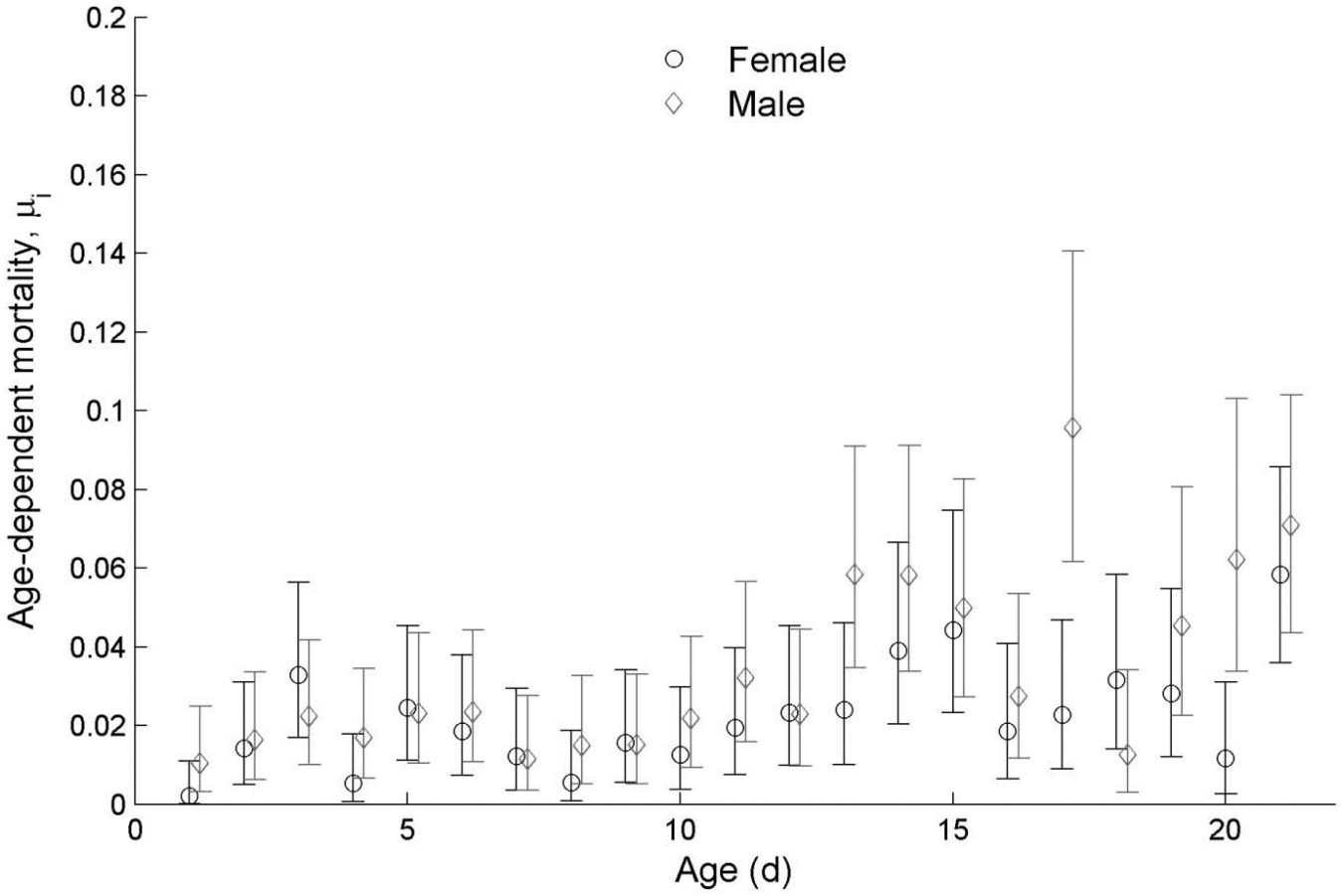

Fig. 3.

Daily mortality estimates (mean and 95% CI) for male and female An. gambiae s.s. based on a temperate-setting mesocosm cohort study with access to nectar-rich plants and nightly blood-feeding opportunities.