# Comparison of Methods for Xenomonitoring in Vectors of Lymphatic Filariasis in Northeastern Tanzania

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*Abstract.*

Monitoring *Wuchereria bancrofti* infection in mosquitoes (xenomonitoring) can play an important role in determining when lymphatic filariasis has been eliminated, or in focusing control efforts. As mosquito infection rates can be low, a method for collecting large numbers of mosquitoes is necessary, for example, gravid traps collected large numbers of *Culex quinquefasciatus* in Tanzania, and a collection method that targets mosquitoes that have already fed could result in increased sensitivity in detecting *W. bancrofti*-infected mosquitoes. The aim of this experiment was to test this hypothesis by comparing U.S. Centers for Disease Control and Prevention (CDC) light traps with CDC gravid traps in northeastern Tanzania, where *Cx. quinquefasciatus* is a vector of lymphatic filariasis. After an initial study where small numbers of mosquitoes were collected, a second study collected 16,316 *Cx. quinquefasciatus* in 60 gravid trap-nights and 240 light trap-nights. Mosquitoes were pooled and tested for presence of *W. bancrofti* DNA. Light and gravid traps collected similar numbers of mosquitoes per trap-night, but the physiological status of the mosquitoes was different. The estimated infection rate in mosquitoes collected in light traps was considerably higher than in mosquitoes collected in gravid traps, so light traps can be a useful tool for xenomonitoring work in Tanzania.

## INTRODUCTION

Lymphatic filariasis (LF) is a disease that, though not fatal, can cause serious health problems. Approximately 40 million people worldwide experience lymphedema, elephantiasis, or urogenital disorders as a consequence of LF infection.[1] Because of several features of this disease, including the lack of an animal reservoir and the availability of safe and effective drugs, LF was targeted for elimination as a public health problem in 1997.[2] Elimination of LF is based on repeated distribution of anthelmintic drugs, treatment of existing cases, and vector control, where appropriate.[3]

To ensure the progress of LF control efforts and to certify elimination, effective monitoring programs must be put in place. Monitoring can be done in one of two ways. First, human blood can be tested for the presence of the parasite. The testing of blood has been facilitated with the development of rapid diagnostic tests.[4] Second, mosquitoes can be collected and tested (xenomonitoring), either through dissection to find the filarial larvae, or through the use of molecular methods to detect the DNA of the filarial worms.[5]

Light traps are commonly used for the collection of mosquitoes for LF xenomonitoring,[6–8] and are usually placed indoors near a sleeping person who is protected with an untreated

mosquito net. Other methods such as human landing catches have been used,[9] but this method is used less often due to ethical concerns.[10]

Gravid traps have been used for the collection of mosquitoes for xenomonitoring, particularly in the United States for monitoring of Western Equine, St. Louis, and West Nile viruses.[11–13] Gravid traps provide oviposition cues and preferentially collect gravid mosquitoes approaching the organic infusion in the pan below the trap. As gravid mosquitoes have typically fed at least once, it is expected that there is a greater chance of collecting infected mosquitoes. Reports have indicated that infection rates in mosquitoes collected in gravid traps was 33 times higher than in mosquitoes collected in dry-ice baited light traps in Delaware.[14] A similar trend was found in New York, where infection rates in mosquitoes collected with gravid traps were 5.7 times higher than in mosquitoes from light traps.[15] Muturi and others[16] used gravid traps in a rice-growing area in Mwea, Kenya, and found that the traps collected several of the primary vectors of *Wuchereria bancrofti* in Africa, namely, *Anopheles arabiensis*, *Anopheles funestus*, and the most numerous in the traps, *Culex quinquefasciatus*. Similarly, Irish and others[17] collected over 400 female *Cx. quinquefasciatus* per night in gravid traps in Ifakara, Tanzania.

The studies described here aimed to compare gravid traps with light traps in an area endemic for LF, both in terms of the numbers of mosquitoes collected and the proportion of mosquitoes infected with *W. bancrofti*. An initial study was conducted at the end of the rainy season (September–October 2011). Because of the fairly small numbers of mosquitoes collected, a second study was conducted during the subsequent rainy season (June–July 2012).

## MATERIALS AND METHODS

### Study sites.

Both the initial study and the secondary study were conducted in Tanga region. The initial study was performed in Masaika and the secondary study was conducted in Vyeru (Figure 1). Masaika is a village of approximately 1,000 inhabitants (S 05.27659, E 38.85017). It is primarily a village of subsistence farmers and has rainy seasons in March–June (heavy rains) and October–November (light rains).[18,19] A detailed description of the village is provided by Rwegoshora and others.[7] In this village, 12 households were recruited for mosquito trapping that was conducted from September 6 to October 14, 2011. Vyeru is a village on Manza Bay (S 04.95754, E 39.13483). The seasons are similar to Masaika, and fishing and subsistence agriculture are both practiced by inhabitants of this village. From this village 10 houses were selected and trapping was conducted between June 27 and July 31, 2012.

In both locations, village leaders were consulted to see if the study would be acceptable. Once their approval was gained, homeowners were approached to see if they would be willing to participate in the study. The locations of consenting households were measured using a GPS unit (Garmin Ltd., Southampton, United Kingdom) to ensure that all participating households were at least 50 m from each other. As a condition of ethical clearance (LSHTM no. 5966, NIMR no. 1242), participating households were given long-lasting insecticide-treated bed nets (Olyset Net; Net Health Ltd., Arusha, Tanzania) before the trial.

### Collection methods.

The model of light trap used, the U.S. Centers for Disease Control and Prevention (CDC) light trap (Bioquip Products, Inc., Rancho Dominguez, CA), was selected because it is the same

model used by the study monitoring the Tanzanian National Lymphatic Filariasis Elimination Program to collect mosquitoes for xenomonitoring. Light traps were placed indoors and were hung from rafters next to the beds.

The gravid traps used were CDC gravid traps (John W. Hock Co., Gainesville, FL), which were shown to be the most effective model for collecting *Cx. quinquefasciatus* in a previous trial.[17] The traps were run on 6 V batteries, which were placed inside the house for security, while the trap was placed outside the house under the eaves. The wire connecting the trap to the battery was passed through the door frame or a small hole in the wall (Figure 2).

Grass infusion was prepared by soaking grass in water for 2 days (approximately 4–5 g of grass per liter). While soaking the grass, the plastic bin was covered to prevent any mosquito oviposition. The grass was strained from the infusion using a sieve before it was added to the gravid traps. Of the grass infusion, 4 L was used per gravid trap.

**Study design.**

The initial study involved 12 households in Masaika at the end of the rainy season in 2011. Each night 10 light traps and two gravid traps were set at the 12 households, with one trap set at each house. Only two gravid traps were used because of the large numbers of *Cx. quinquefasciatus*, based on collections from previous experiments.[17] The position of the traps was determined using a randomized Latin square design. The traps were set twice weekly for 6 weeks, resulting in 24 trap-nights for the gravid traps, and 120 trap-nights for the light traps.

In Vyeru, study was conducted during the rainy season to increase total catch and better determine the difference between the two collection methods. Eight light traps and two gravid traps were set each night over 30 nights, resulting in 240 trap-nights for light traps and 60 trap-nights for gravid traps.

In both studies, traps were set between 5:00 and 7:00 PM and were collected the following morning between 06:00 and 07:30 AM. Trap nets were returned to the laboratory and collected mosquitoes were killed in a −20°C freezer. Mosquitoes were identified to species, using available keys.[20–23] After identification, mosquitoes were individually placed in Eppendorf tubes (1.5 mL). Each tube contained a small amount of anhydrous calcium sulfate (W. A. Hammond Drierite Company Ltd., Xenia, OH), topped with cotton wool, to prevent the growth of mold. Any nights in which either the trap battery/trap failed or ants were present in the trap were discounted and not included in the final analysis.

**Detection of *W. bancrofti* in collected mosquitoes.**

Female *Cx. quinquefasciatus* mosquitoes from Masaika were tested individually for the presence of *W. bancrofti* DNA using the method of Chambers and others,[24] with the exception that BlueJuice loading buffer (Invitrogen, Carlsbad, CA) and ethidium bromide were used instead of GelRed (Biotium, Hayward, CA). In brief, DNA extractions using the Qiagen DNeasy Kit involved homogenizing the individual mosquitoes in 200 µL of phosphate-buffered saline. The AL buffer (200 µL) and 20 µL of proteinase K were added, and the homogenate incubated at 70°C for 10 minutes. An additional 20 µL of proteinase K was then added followed by incubation at 56°C for 1 hour. This was then followed by a centrifugation 14,500 $g$ for 5 minutes to pellet the mosquito debris. The supernatant was transferred into new Eppendorf tube and mixed with 200 µL of 98% ethanol, and the entire sample loaded into DNeasy mini column. This

was followed by centrifugation at 9,000 *g* for 1 minute where DNA bound to the DNeasy column membrane and other materials passed through. The DNA was then washed three times using two grade washing buffers to remove any contaminants, and thereafter eluted with 250 mL of elution buffer and stored at –20°C until used for polymerase chain reaction (PCR) amplifications.

Positive samples were retested to confirm presence of *W. bancrofti* DNA. Each run contained a negative control (double distilled water) and a positive control (DNA extract from mosquitoes fed on a patient with LF, previously tested positive for microfilaremia).

As the numbers of *Cx. quinquefasciatus* females from Vyeru were considerably higher than those from Masaika, mosquitoes were pooled in groups of 25 before DNA extraction. The same extraction method that was used for the individual mosquitoes from Masaika was used for the pools from Vyeru. Females of all physiological stages were used in the pools. Because of limited reagents, a subsample of pools to be tested was selected using a random number generator. The *Anopheles* mosquitoes were tested individually. The DNA was extracted in the same manner as the mosquitoes from Masaika, and was also screened using quantitative PCR with Roche short hydrolysis universal probes substituted with locked nucleic acids, which allow greater specificity than standard PCR. Probes and corresponding primers were designed using the Roche ProbeFinder software to amplify genes to detect *W. bancrofti* (GenBank accession no. AY297458), *Cx. quinquefasciatus* (GenBank accession no. L34351.1), and the endosymbiotic bacterium of *Cx. quinquefasciatus*, *Wolbachia pipientis w*Pip strain (GenBank accession no. AF020061.1). Synthetic long oligonucleotides of the amplified product were generated and 10-fold serial dilutions were carried out to produce a standard curve of gene copies per microliter for all genes assayed. Ten microliter PCR reactions containing 0.5 μM forward and reverse primers and 0.1 μM hydrolysis probe (final concentrations) were added to Roche FastStart essential DNA probes master mix. A Roche LightCycler 96 System was used to first preincubate the reactions at 95°C for 5 minutes, followed by 45 cycles of 60°C for 30 seconds and 95°C for 10 seconds. Two independent assays were run each with three replicates/sample and assays and included negative (no template) and positive controls. *Wuchereria bancrofti* infection levels were normalized to the host *Cx. quinquefasciatus* gene to generate a mean *Wuchereria*:*Culex* gene ratio for each DNA extract.

**Statistical analysis.**

The numbers of female *Cx. quinquefasciatus* collected per trap-night were checked for normality. If the data followed a negative binomial distribution, a negative binomial regression model was used to compare the means. The trap day, trap location, and trap type were tested using univariate analyses. Those having an effect on the numbers of female *Cx. quinquefasciatus* collected at the 10% level ($P < 0.1$), as tested using Wald tests, were retained for multivariable analysis. The final model included all variables significant at the 5% level ($P < 0.05$) when adjusted for other factors. All analyses were conducted using Stata 11.0 (Stata Corporation, College Station, TX).

For comparisons of the infection rates in individually analyzed female *Cx. quinquefasciatus* from Masaika and *Anopheles gambiae* from Vyeru, $\chi^2$ tests were used. As the numbers of infected mosquitoes were low, Fisher exact tests were used. For pooled *Culex* mosquitoes from Vyeru, infection rates were calculated using Poolscreen 2.0 software, providing maximum likelihood estimates for the rate of infection.[25]

**First study at the end of the dry season in Masaika.**

Over the course of the trial, 432 mosquitoes were collected. As some of the mosquitoes were damaged by the fans in the traps, a mosquito was counted if the thorax was present (which excludes excised heads and abdomens). Mosquitoes collected are shown in Table 1. *Culex quinquefasciatus* was the only LF vector collected in any of the traps. No *Anopheles* mosquitoes were collected. The median numbers of female *Cx. quinquefasciatus* and *Cx. cinereus* from the two types of trap are shown in Table 2. Of the 150 female *Cx. quinquefasciatus* collected in the gravid traps, 121 (81%) were gravid. Of the 12 female *Cx. quinquefasciatus* collected in light traps, three (25%) were gravid.

The numbers of females of *Cx. quinquefasciatus* were not distributed normally, so a negative binomial regression model was used to analyze the data. The trap day and trap type were retained in the final model. The gravid traps collected significantly more female *Cx. quinquefasciatus* than the light traps ($z = 10.70$, $P < 0.001$).

Molecular methods were used to test for the presence of *W. bancrofti* DNA. All of the female *Cx. quinquefasciatus* and unidentified mosquitoes were tested. Two *Cx. quinquefasciatus* specimens tested positive for *W. bancrofti* were collected using gravid traps. Of the *W. bancrofti* positive *Cx. quinquefasciatus*, one of the mosquitoes was unfed at the time of collection, the other was gravid. Since only 150 *Cx. quinquefasciatus* were tested, this resulted in an infection rate of 1.3%.

**Second study during the rainy season in Vyeru.**

During the study conducted in Vyeru in the rainy season, 16,471 intact female mosquitoes were collected from light and gravid traps (Table 1). The *Cx. quinquefasciatus* were further separated by physiological status. As is shown in Table 3, the majority of *Cx. quinquefasciatus* collected in light traps were unfed, while the majority of *Cx. quinquefasciatus* collected in gravid traps were gravid.

The total numbers of *Cx. quinquefasciatus* collected in the two trap types were compared using a negative binomial regression model that included whether it had rained, the house where collections were made, and the trap type. The total collections of *Cx. quinquefasciatus* in the two trap types were not significantly different ($z = 1.19$, $P = 0.23$).

A total of 110 pools of 25 female *Cx. quinquefasciatus* from light traps and 113 pools of 25 female *Cx. quinquefasciatus* from gravid traps were tested for the presence of *W. bancrofti* DNA using quantitative PCR. Of the pools from light traps, 15 tested positive, while only one of the pools from gravid traps tested positive (Figure 3). Using the maximum likelihood estimates, 0.6% (0.31–0.99%) of *Cx. quinquefasciatus* would be expected to be positive for presence of *W. bancrofti* in mosquitoes from light traps, while only 0.03% (0.001–0.2%) of mosquitoes collected in gravid traps would be expected to be positive. All samples were positive for the endosymbiotic bacterium *W. pipientis w*Pip strain, native to *Cx. quinquefasciatus*, confirming the mosquito species status. Pooling 25 mosquitoes for each DNA extract prevented a quantitative comparison of *Wuchereria* infection levels in individual mosquitoes from the two types of traps. However, statistical analysis using a $\chi^2$ test to compare the two infection rates, revealed a significant difference between the types of traps ($P = 0.0004$).

None of the *Anopheles* tested positive for the presence of *W. bancrofti* DNA.

DISCUSSION

This study conducted two similar studies in the Tanga region of Tanzania, where both *Anopheles* and *Culex* vectors are involved in transmission of *W. bancrofti*.[18] In these studies, the primary outcomes measured were the numbers of mosquitoes collected and the proportion of mosquitoes infected.

In Masaika, significantly more *Cx. quinquefasciatus* were collected in gravid traps, whereas in Vyeru, the numbers collected in light and gravid traps were not significantly different. One of the potential reasons for this difference in results is the seasons in which collections were conducted. In Masaika, collections were made at the end of the rainy season, and rain did fall over the course of the study. As gravid traps attract mosquitoes by presenting potential breeding sites, their attractiveness may be decreased when alternative sites are present. Therefore, gravid traps might be more attractive during the dry season, when fewer mosquitoes are present and fewer breeding sites are available to gravid mosquitoes. The study in Vyeru was conducted during the rainy season, when many natural oviposition sites were available. The number of breeding sites available might have reduced the attractiveness of (and therefore the number collected in) gravid traps. These effects might also be affected by the difference in study site.

Another important factor to take into consideration is the difference in position of the two traps. The light traps were located indoors, next to the beds where humans were sleeping. The gravid traps were placed outdoors, under the eaves of the house. There are two reasons why this may have affected the numbers of mosquitoes collected. The first is the fact that pyrethroid-treated nets were distributed before the collection of mosquitoes, as required for ethical approval. Long-lasting Olyset nets have been shown to have an effect on the numbers of mosquitoes entering a house.[26] However, Magbity and others[27] found that numbers of *An. gambiae* s.s. collected in CDC light traps next to treated and untreated nets were not significantly different. Furthermore, although nets were distributed, it was clear from observations while setting the traps that not all nets were being used. Second, although in most cases, because of the small size of most of the houses used, the distance between the locations used for the two types of traps was not more than 5 m, the two traps were nevertheless placed in different climatic conditions, which may have affected the trap catches. This is acceptable, because the goal of this project was not to compare the trap efficacy as such, but rather their potential for xenomonitoring in a typical surveillance program.

A difference in infection rates was not found in Masaika, although infected mosquitoes were only found in gravid traps. However, with only 10 mosquitoes collected in light traps, it was not possible to determine whether the infection rates of mosquitoes collected with each method were different. In Vyeru, however, large numbers of mosquitoes were pooled and a significantly higher proportion of pools from light traps tested positive for *W. bancrofti* DNA than in pools from gravid traps.

The initial hypothesis of this study was that as gravid traps collect greater numbers of mosquitoes that have had at least one blood meal, a greater number of infected mosquitoes would be found in gravid traps. Although it is difficult to explain why there were more infected mosquitoes in pools from light traps, there are a few potential explanations. First, blood-fed mosquitoes were included in pools from both traps, but considerably higher numbers of blood-fed mosquitoes were collected with light traps. The presence of microfilariae in an undigested

infective blood meal could result in a positive pool. In a comparison of infection rates between *Anopheles punctulatus* collected using light traps and human landing catches in Papua New Guinea, it was proposed that infection rates were higher in light trap catches because of the increased proportion of blood-fed mosquitoes in light trap catches.[6] This presents an important question for xenomonitoring in general: how representative is a PCR-positive mosquito of *W. bancrofti* prevalence in a community? On one hand, if the xenomonitoring is intended to show whether transmission is possible in a community, the presence of a positive mosquito indicates that a mosquito has taken a blood meal from microfilaremic human in the immediate vicinity. On the other hand, it is considerably more difficult to estimate the prevalence in an entire community based on light traps that are placed in houses of individuals, who may or may not be infective to mosquitoes. Second, the fact that gravid traps were placed outdoors may result in collecting a more representative sample of all mosquitoes in a community, as mosquitoes entering the trap may have exited multiple houses, or bit people in multiple locations. However, *Cx. quinquefasciatus* are not exclusively anthropophilic,[28] so gravid traps may include mosquitoes that have fed on birds or other animals, thereby decreasing the likelihood of infection. Sampling from inside houses may increase the likelihood of collecting anthropophilic mosquitoes. As *W. bancrofti* is only present in humans, any trapping bias toward anthropophilic mosquitoes is likely to result in an increased chance of collecting PCR-positive mosquitoes.

*Culex quinquefasciatus* are not the only vectors of *W. bancrofti* in Tanzania. *Anopheles gambiae* and *An. funestus* have also been found to play an important role in transmission.[18] However, gravid traps baited with grass infusion are considerably less effective in collecting *Anopheles* mosquitoes than light traps.[16] Depending on the local vectors of filariasis, light traps may be a more valuable sampling method, as they can collect not only *Cx. quinquefasciatus* that are more likely to be infected (as in this study), but also higher numbers of *Anopheles* vectors. However, even in Tanzania, where *Anopheles* vectors transmit the larvae of *W. bancrofti*, the importance of *Cx. quinquefasciatus* is increasing.[8] The proportion of microfilariae ingested by *Cx. quinquefasciatus* that reach the infective L3 stage increases with declining numbers of microfilariae ingested (limitation), whereas the opposite is true for *Anopheles* vectors (facilitation).[29] As mass drug administration reduces microfilarial intensity,[30] it is likely that *Cx. quinquefasciatus* will be a more important vector than *Anopheles* as the goal of elimination is approached.

In conclusion, the two trapping experiments described herein provide further evidence of an inherent bias that is typical when using different collection methods. The gravid traps collected significantly more *Cx. quinquefasciatus* in Masaika at the end of the rainy season, but there was no significant difference when the trapping was conducted in the middle of the rainy season in Vyeru. This may indicate an effect of the presence of natural alternative breeding sites on the efficacy of the gravid trap, which essentially provides an artificial breeding site. In Masaika, there was no significant difference in the proportion of infected mosquitoes collected, which was likely due to the small numbers of mosquitoes collected. In Vyeru, where a larger sample size was used, a significantly higher proportion of infected *Cx. quinquefasciatus* were collected in light traps than in gravid traps. It seems that the light trap is more adapted toward xenomonitoring efforts in Tanzania, particularly as it is also used to collect *Anopheles* vectors, but the gravid trap may be useful where *Cx. quinquefasciatus* is the only vector.

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FIGURE 1. A map of northeastern Tanzania showing the study villages Masaika and Vyeru. This figure appears in color at www.ajtmh.org.

FIGURE 2. A gravid trap placed outside a house in Masaika, Tanzania. This figure appears in color at www.ajtmh.org.

FIGURE 3. *Wuchereria bancrofti* infection levels using quantitative polymerase chain reaction (qPCR) in pooled *Culex quinquefasciatus* DNA extracts collected from light (blue) and gravid (red) traps in Vyeru, Tanga Region of northeastern Tanzania. Roche universal probes and associated primers were used to detect *Wuchereria* infection levels, which were normalized to detected levels of a *Cx. quinquefasciatus* host gene. This figure appears in color at www.ajtmh.org.

TABLE 1

Species of mosquito collected in CDC light traps and CDC gravid traps in Masaika (September–October 2011) and Vyeru (June–July 2012), northeastern Tanzania

| Species | Masaika | | Vyeru | |
|---|---|---|---|---|
| | Light (N = 24) | Gravid (N = 120) | Light (N = 207) | Gravid (N = 56) |
| *Culex quinquefasciatus* | 12 | 150 | 12,650 | 3,666 |
| *Culex cinereus* | 40 | 173 | 14 | 2 |
| *Mansonia uniformis* | 1 | 0 | 40 | 8 |
| *Lutzia tigripes* | 1 | 1 | 11 | 17 |
| *Mansonia africana* | 1 | 0 | 16 | 2 |
| *Anopheles gambiae* s.l. | 0 | 0 | 12 | 0 |
| *Culex macfiei* | 0 | 0 | 0 | 9 |
| *Culex univitattus* | 0 | 0 | 8 | 0 |
| *Culex decens* | 1 | 2 | 3 | 1 |
| *Culex poicilipes* | 0 | 0 | 3 | 1 |
| *Culex vansomereni* | 0 | 0 | 2 | 0 |
| *Stegomyia* spp. | 0 | 0 | 1 | 1 |
| *Coquillettidia metallica* | 0 | 0 | 2 | 0 |
| *Stegomyia aegypti* | 1 | 1 | 0 | 0 |
| *Culex bitaeniorhynchus* | 0 | 0 | 1 | 0 |
| *Culex thalassius* | 0 | 0 | 1 | 0 |
| *Mimomyia mimomyiaformis* | 1 | 0 | 0 | 0 |
| *Culex nebulosus* | 0 | 1 | 0 | 0 |
| *Culex insignis* | 1 | 0 | 0 | 0 |
| Unidentified | 6 | 25 | 0 | 0 |

CDC = U.S. Centers for Disease Control and Prevention.

TABLE 2

Total, median, IQR, and range of female *Culex quinquefasciatus* and *Culex cinereus* from gravid and light traps in Masaika, Tanga, Tanzania, which were set twice weekly in 12 houses between September 6 and October 14, 2011

| | n | *Cx. quinquefasciatus* | | | | *Cx. cinereus* | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | Total | Median | IQR | Range | Total | Median | IQR | Range |
| Light traps | 120 | 12 | 0 | 0–0 | 0–2 | 40 | 0 | 0–0 | 0–6 |
| Gravid traps | 24 | 150 | 5 | 1–8.5 | 1–19 | 173 | 0 | 0–5.5 | 0–66 |

IQR = interquartile range.

TABLE 3

Median and IQR of female *Culex quinquefasciatus* collected in light and gravid traps in Vyeru, Tanzania, showing physiological stage of collected mosquitoes and total numbers

| | Light trap (N = 207) | | Gravid trap (N = 56) | |
|---|---|---|---|---|
| | Median | IQR | Median | IQR |
| Unfed | 36 | 17–68 | 2 | 1–4 |
| Blood-fed | 3 | 1–6 | 0 | 0–0 |
| Semigravid | 0 | 0–1 | 0 | 0–0 |
| Gravid | 3 | 1–7 | 53.5 | 32–80 |
| Total | 44 | 24–84 | 57 | 35.5–83.5 |

IQR = interquartile range.

**Figure 1**

**Figure 2**

**Figure 3**