An assessment of mosquito collection techniques for xenomonitoring of anopheline-transmitted Lymphatic Filariasis in Ghana

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Abstract
Monitoring vectors is relevant to ascertain transmission of lymphatic filariasis (LF). This may require the best sampling method that can capture high numbers of specific species to give indication of transmission. Gravid anophelines are good indicators for assessing transmission due to close contact with humans through blood meals. This study compared the efficiency of an Anopheles gravid trap (AGT) with other mosquito collection methods including the box and the Centres for Disease Control and Prevention gravid, light, exit and BioGent-sentinel traps, indoor resting collection (IRC) and pyrethrum spray catches across two endemic regions of Ghana. The AGT showed high trapping efficiency by collecting the highest mean number of anophelines per night in the Western (4.6) and Northern (7.3) regions compared with the outdoor collection methods. Additionally, IRC was similarly efficient in the Northern region (8.9) where vectors exhibit a high degree of endophily. AGT also showed good trapping potential for collecting Anopheles melas which is usually difficult to catch with existing methods. Screening of mosquitoes for infection showed a 0.80–3.01% Wuchereria bancrofti and 2.15–3.27% Plasmodium spp. in Anopheles gambiae. The AGT has shown to be appropriate for surveying Anopheles populations and can be useful for xenomonitoring for both LF and malaria.

Introduction
Lymphatic filariasis (LF) is a neglected tropical disease that causes debilitating, acute and chronic morbidities in affected individuals. It is caused by three mosquito-borne parasitic worms: Wuchereria bancrofti, which accounts for 90% of cases recorded globally (Ottesen, 2006); Brugia malayi and Brugia timori accounting for the remaining 10%. LF is present in over 80 countries in the Americas, Asia, the Pacific and Africa (Molyneux et al., 2003). It is estimated that 120 million of the world’s population is infected, while 40 million suffer from disabilities and psychological trauma due to stigmatization (Brady, 2014; Ichimori et al., 2014; WHO, 2015). It is transmitted by mosquitoes belonging to the Aedes, Anopheles, Culex, Mansonia and Ochlerotatus genera (de Souza et al., 2012). In Africa, where W. bancrofti is the parasite responsible for LF, anophelines (Anopheles gambiae s.l. complex and Anopheles funestus) are the main vectors in rural areas across the continent while culicines (Culex quinquefasciatus) are the primary vectors in urban areas in eastern and southern parts of Africa (Pederson, 2008). More recently, Mansonia mosquitoes have been incriminated as potential vectors in rural Africa as well (Ughasi et al., 2012).

The Global Programme to Eliminate Lymphatic Filariasis (GPELF) was launched in 2000, with the primary goal to interrupt LF transmission through annual mass drug administration (MDA) with albendazole in combination with either ivermectin in Africa or diethylcarbamazine in areas outside Africa (Ottesen, 2006). The GPELF has achieved great successes since its inception, with the elimination of LF in Cambodia, Cook Islands, Maldives, Niue, Sri Lanka, Togo and Vanuatu (Budge, 2014; WHO, 2016, 2017). In addition, 13 countries are now in the post-elimination phase.

According to the WHO guidelines, transmission assessment surveys (TAS) support the decision to stop MDA (WHO, 2011) based on microfilaria (mf) prevalence <1% or antigen prevalence <2% (Stolk et al., 2003). The tools available for transmission assessment include; immunochromatographic test (ICT) [such as filarial test strip (FTS)], ELISA, polymerase chain reaction (PCR) and mf detection by microscopy (WHO, 2011). However, these tools require blood collection from large numbers of community volunteers. Further, the cross-reactivity of Loa loa and W. bancrofti reported by Wanjii et al. (2015) in Cameroon, questions the reliability of ICT especially in parts of Central Africa where these parasites are co-endemic. Meanwhile, monitoring vectors for the presence of parasite DNA (xenomonitoring) is an important assessment tool for LF elimination programmes, with the advantage that it provides a real-time estimate of microfilaria in the community members (Okorie and de Souza, 2016). Mosquito surveillance studies have shown to be useful in assessing the transmission of LF in the Pacific (Chanteau et al., 1994; Burkot and Ichimori, 2002; Farid et al., 2007). Furthermore,
studies in Africa show that xenomonitoring can provide valuable information to the LF elimination programme (Chanteau et al., 1994; Boakye et al., 2004; Ughasi et al., 2012; de Souza et al., 2014). The traditional methods for xenomonitoring include dissection vectors for the different developmental stages of the worm. However, as microfilariae (mf) prevalence in human populations becomes low due to MDA, the time and cost involved in processing such large numbers remain a challenge (Burkot and Ichimori, 2002). In this instance, molecular xenomonitoring, a method which allows for processing samples within a shorter time with high precision will improve sample processing (Derru et al., 2017). Additionally, targeting epidemiologically relevant mosquitoes, i.e. older mosquitoes (blood fed, gravid or parous mosquitoes) will further enhance the chances of detecting parasite DNA (Springer et al., 2016). Gravid traps have shown to be useful for such purposes (Mboera et al., 2000). The Centres for Disease Control and Prevention (CDC) gravid traps are routinely used for surveying culicine populations (Williams and Gingrich, 2007; Facchinelli et al., 2008), however, the attractive oviposition substrate is not effective in collecting anophelines. Recent work by Lindh et al. (2016) point to the potential of the Anopheles gravid trap (AGT) (OviART) in collecting gravid Anopheles mosquitoes. Other mosquito collection tools for monitoring mosquito populations include indoor and outdoor collection methods (e.g. the human landing catches, pyrethrum spray catches (PSC), aspiration of resting mosquitoes, exit traps (ET), barrier nets, box gravid trap (BOX), CDC-light trap (LIT), and the BioGents-sentinel trap). The traps exploit different mosquito behaviours, such as feeding and resting, and habitats with varying sensitivities.

The aim of our study was to assess the efficiency of the AGT for xenomonitoring purposes in two LF endemic areas in Ghana – the coastal Western region and the Northern region. The study evaluated the mosquito composition, density and physiological state of mosquitoes collected from this trap compared with five other collection methods, as well as W. bancrofti and Plasmodium spp. DNA positivity in the mosquitoes collected.

Materials and methods

Study sites

Our study was conducted in villages across the Western Region (Akonu, Agyan and Asemko) and Northern Region (Dugli and Sekyerekura) of Ghana (Fig. 1). Akonu and Agyan are neighbouring communities located in the Nzema East district while Asemko is in the Ahanta West district. Dugli and Sekyerekura are neighbouring communities in the Bamboni district. The major vectors of LF in the Northern savanna region are An. gambiae, Anopheles arabiensis and Anopheles funestus while in the coastal areas, the predominant vectors are A. gambiae s.s, Anopheles melas and An. funestus (de Souza et al., 2010). The Northern region is characterized by a rainy season which occurs between May and September and a dry season between December and April with temperatures as high as 40 °C. The Western region has a wet season that spans from April to November and a dry season between December and March.

Epidemiological survey

An epidemiological survey was conducted to determine the LF prevalence in Agyan (May 2016), Akonu and Asemko (January 2017), Dugli and Sekyerekura (October 2017). Finger-prick blood was collected from volunteers aged 16 years and above and tested using the FTS (Alere) to detect circulating filarial antigen. Positive individuals were then followed up for the presence of microfilariae through the collection of blood between 10 pm and 2 am. This coincides with the peak time of mf in peripheral blood. Blood samples were screened by microscopy using the counting chamber technique described by Agbolade and Akinboye (2005).

Mosquito collection methods

The AGT was designed and described by Dugassa et al. (2013). It was made of a rectangular basin measuring 45 cm × 33 cm × 11.5 cm (length × width × height), with a 4 cm hole on the side and 6 L rectangular basin. An open plastic tube (collection chamber) was inserted into the hole and the other opening of the tube was sealed with fibre glass netting to prevent trapped mosquitoes from escaping. The tube was placed and secured halfway into an aluminium collapsible pipe. The flexible tube was connected to a 12 V fan that provided suction on the water surface. The efficiency of AGT was compared with two gravid traps; BOX-the Box gravid trap (by BioQuip, Rancho Dominguez, CA) and CDC-gravid trap (Model 1712 from John W. Hock Company), and LIT (by UPL limited) for outdoor collections. The AGT was also evaluated against the BGS-BioGents-sentinel, ET-Exit traps, IRC-Indoor resting collection and the PSC-pyrethrum spray catches which were used to collect mosquitoes indoor. The BGS (BioGents) was baited with the Anopheles odour lure. The LIT was baited with cotton wool soaked in 1-Octen-3-ol. The IRC were done using a battery-powered aspirator (Vazquez-Prokopec et al., 2009), with the interior ceiling, walls and any hanging clothing aspirated. The AGT, BOX, CDC, ET, LIT and BGS were set up at 6 pm and removed the following morning at 6 am. PSCs were conducted between 5:30 am and 6 am in selected houses in the Western region. Whereas, the IRCs were conducted from 5:30 am to 8:30 am in all households in the villages in the Northern region. Water from larval habitats was used in all the
gravid traps. Only valid collection nights (nights where all the traps worked through the night) were used in comparisons. The data collected on days where batteries of one or more of the traps failed were excluded.

**Western region mosquito collection**

The AGT was compared with CDC, BOX, LIT, ET and PSC in the three Western region villages. Mosquito collections were conducted in March and May 2017 with a total of 26 collection nights each for AGT, BOX, CDC and LIT; 18 for ET and 37 for PSC in 13 consecutive nights. The four outdoor collection methods (AGT, CDC, BOX and LIT) were rotated among selected locations within the densely populated sections of the villages and larval habitats. The two indoor methods (ET and PSC) were done in randomly selected rooms with at least one sleeper.

**Northern region mosquito collection**

The AGT was compared with the BOX, BGS and IRC in the Northern region. Mosquito collections were conducted in October 2017 with 12 collection nights for each trap type. The three outdoor traps (AGT, BOX, BGS) were rotated among selected locations within each village. Each location consisted of a family compound and IRCs were conducted in all rooms which were occupied by a sleeper the night before (the number of rooms per compound ranged 1–8). The mean number of mosquitoes per room is presented for each location (Fig. 5).

**Mosquito processing**

The collected mosquitoes were identified morphologically using keys by Gillies and Coetee (1987). Mosquitoes collected from the Western region were scored based on abdominal status – fed, unfed or gravid. A proportion of the mosquitoes collected from the Western region were dissected and ovaries removed and dried to determine parity based on ovary tracheation as described by Detinova (1962). Mosquito legs were used for molecular identification of members of the *An. gambiae* s.l. complex, based on restriction fragment length polymorphism described by Fanello et al. (2002). Mosquitoes from the Northern region were only identified using morphological features.

**Wuchereria bancrofti and Plasmodium detection in mosquitoes**

The anopheline mosquitoes that were in good condition (not damaged) were screened for *W. bancrofti* and *Plasmodium* spp. DNA, in pools of up to 5 mosquitoes based on trap type, species and location. The heads and thoraces were screened separately from the abdomens. Genomic DNA (gDNA) was extracted from the mosquito pools, using the Livak extraction method (Livak, 1984). To determine the presence of *W. bancrofti* DNA, the ITS1 gene in the 18S and 5.8S subunits of the rRNA from filarial worms was amplified as described by Jiménez et al. (2011). For the identification of *Plasmodium* spp. DNA, the COX-1 gene was amplified in a single step PCR as described by Echeverry et al. (2017).

**Data analysis**

Morphological and molecular identification showed no differences in species composition between villages of the same region, therefore, data were combined for statistical analysis using SPSS (IBM24). To evaluate the trapping efficiency of AGT, a Bonferroni posthoc analysis of variance (ANOVA) was performed to compare the mean number of *Anopheles* mosquitoes caught by each method, per night.

Trapping efficiency was also evaluated by comparing an estimated mean number of *Anopheles* that were likely to have taken a blood meal in their lifetime since these are the target population for xenomonitoring. The proportion of mosquitoes that were unfed but parous, blood fed and gravid mosquitoes collected in each trap was estimated and multiplied by the mean catch per collection/night to get the estimated number that had previously taken a blood meal.

The prevalence of *W. bancrofti* and *Plasmodium* spp. DNA in mosquitoes was estimated using the PoolScreen software 2.0 (Katholi et al., 1995) and the maximum likelihood estimates reported with 95% confidence interval (CI).

### Results

#### LF infection prevalence

Filarial antigen prevalence ranged 12.2–27.9% in the study villages and microfilaria prevalence ranged 1.5–3.8% (Table 1).

### Mosquito composition

#### Western region

A total of 1417 mosquitoes was collected in the Western region. Morphological identification of collected mosquitoes showed that 4.6% were *Aedes* spp, 36.8% were *A. gambiae* s.l., 58.4% were *Culex* spp and 0.2% were *Mansonia* spp. There was no significant difference in the species composition across the three villages. The largest proportion of mosquitoes caught using the outdoor collection methods (AGT, BOX, CDC and LIT) were *Culex* whereas, the largest proportion of the total catch in ET and PSC were *Anopheles* (Fig. 2). Molecular characterization of the 442 *An. gambiae* s.l. collected from the villages in the Western region, show that 44.0% were *An. gambiae* s.s., 43.8% were *An. melas*, while 12.1% did not amplify. Whereas in the Northern region, 55.1% of 78 *Anopheles* were identified as *An. gambiae* s.s. however, 44.9% did not amplify with the *An. gambiae* complex primers used (primers included; *An. arabiensis*, *An. gambiae* and *An. melas*). These proportions did not differ

### Table 1. Estimated CFA and mf prevalence in the five villages

| Region  | Community | Number tested for CFA | CFA prevalence (%) | Estimated village microfilaria prevalence (%) |
|---------|-----------|-----------------------|--------------------|-----------------------------------------------|
| Western | Agyan     | 195                   | 23.0               | 2.2                                           |
| Western | Akonu     | 93                    | 27.9               | 1.5                                           |
| Western | Asemko    | 115                   | 12.2               | 1.7                                           |
| Northern| Dugu      | 78                    | 19.2               | 2.6                                           |
| Northern| Sekyerekura| 79                | 15.2               | 3.8                                           |

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between the indoor and outdoor collection methods ($\chi^2 = 0.16, P = 0.7$).

**Northern region**

A total of 771 mosquitoes was collected in the Northern region. Of the 555 mosquitoes collected indoors, 473 (85.2%) were *An. gambiae* s.l, and 68 (12.2%) were *An. funestus*, 10 (1.8%) were other *Anopheles* spp. including *Anopheles rufipes* and *Anopheles coustani* and 4 (0.7%) were *Culex* spp (Fig. 3).

**Comparison of the trapping efficiency by mosquito composition and density**

In the Western region, AGT caught the highest mean number of anophelines per collection night (4.62, 95% CI 0.49–8.74) followed by PSC (3.05, 95% CI 1.85–4.26) and the least being CDC (0.15, 95% CI 0.01–0.30). Pairwise comparisons show that the differences in the mean catch per collection night of anophelines was only significant for AGT when compared with CDC, $F_{(5, 153)} = 2.959, P = 0.014$ (Fig. 4).

In the Northern region, the indoor resting collections had the highest mean number of anophelines per collection night (8.86, 95% CI 4.13–13.59) followed by AGT (7.33, 95% CI 0.83–13.83) with BGS catching the least (0.58, 95% CI 0.01–1.16). The observed differences in the mean number of anophelines was statistically significant between IRC and BGS but not AGT and BOX ($F_{(3, 44)} = 4.808; P < 0.01$) (Fig. 5). However, the differences in mean between AGT and BGS, AGT and BOX or BGS and BOX were not statistically significant.

**Trapping efficiency for mosquitoes that have previously taken a blood meal**

Estimating the number of mosquitoes that were likely to have previously taken a blood meal is important for xenomonitoring. These mosquitoes include those that were caught bloodfed, gravid or unfed and parous. The parity dissections were only conducted in the Western region study. The results show that AGT caught the highest mean number of *An. gambiae* s.l. previously exposed to at least one blood meal. PSC was second with CDC collecting the least number per collection night. As expected, the gravid traps caught a high proportion of gravid *An. gambiae* s.l. while the indoor collections caught a high proportion on bloodfed *An. gambiae* s.l. (Table 2).

**Infection prevalence in mosquitoes**

Pools of heads and thoraces were split from abdomens and screened separately for *W. bancrofti* and *Plasmodium* spp DNA. The results are presented in Table 3. In the Western region, none of the head/thorax pools were positive for either. However, *W. bancrofti* and *Plasmodium* spp. DNA were detected in pools of mosquito abdomens. A 0.80% and 2.15% prevalence of *W. bancrofti* and *Plasmodium* spp. was observed in the *An.
gambiae collected from the Western region. These included mosquitoes (i.e. 3 pools of 15 mosquitoes) that had been captured using PSC (the only method that had mosquitoes that were positive for W. bancrofti). The Plasmodium positives pools were obtained from 3, 4 and 1 pool of 14, 11 and 2 mosquitoes that were caught in AGT, BOX and ET, respectively. The Plasmodium spp. positives were recovered from the pools that contained unfed, bloodfed and gravid Anopheles mosquitoes, indicating that, immature stages of the parasite were present in the midgut. Unsurprisingly, the W. bancrofti DNA positive pools were all from those that were bloodfed. Screening of pools of Mansonia show a prevalence of 3.57% (95% CI 0.11–17.08%) Plasmodium spp. and 3.86% (95% CI 0.12–18.45) of W. bancrofti DNA. In the Northern region, we observed a higher overall prevalence of W. bancrofti at 3.01% (95% CI 1.60–5.05) and Plasmodium spp. at 3.27% (1.78–5.40). The W. bancrofti positive pools were from 2, 3 and 7 pools of 5, 9 and 29 for AGT, BOX and IRC, respectively. While that of Plasmodium spp. were from 1 to 11 pools of 5 and 52 mosquitoes that were captured using BOX and IRC, respectively. In addition, a small number of head/thorax pools were positive for W. bancrofti (0.3%, 95% CI 0.01–1.5) and Plasmodium spp. (0.9%, 95% CI 0.2–2.5), indicating the developing stage of filarial worms were present in the thorax, and the infective stage sporozoites were present in the salivary glands. The positive mosquitoes were collected from IRC and AGT and these mosquitoes were An. gambiae and Culex spp.

Discussion
Monitoring parasite infection in vector populations can be used to assess transmission as well as infection in the human population (Boakye et al., 2004; Ughasi et al., 2012; de Souza et al., 2014).
This study reports on the first evaluation of an anopheline mosquito collection method for monitoring LF and malaria in vectors in Ghana. In the Western region, about 40% of the total *An. gambiae s.l.* collected were *An. melas*. *Anopheles melas* is usually associated with mangroves, which were present in the study communities. Tuno *et al.* (2010) also reported on the abundance of *An. melas* in the western coast of Ghana. In the Northern Region, the *Anophelines* sampled included *An. coustani*, *An. funestus*, *An. rufipes* and *An. gambiae s.l.* which are also implicated in lymphatic filariasis transmission (Tabue *et al.*, 2017). These *Anophelines* species have been reported to exhibit ‘limitation’ which favours transmission even when mf prevalence is low (McGreevy *et al.*, 1978; Bryan, 1990; Amuzu *et al.*, 2010).

![Fig. 5. Plots of the mean number of anophelines caught by each method per night in the Northern region (*P* < 0.05). Bars with identical letters are not significantly different from each other. Error bars show standard error of the mean.](https://doi.org/10.1017/S0031182018000938)

### Table 2. Estimated mean number of *A. gambiae s.l.* from the Western region which have previously taken a blood meal

| Collection method | Mean catch* (±S.E.) | Proportion unfed nulliparous | Proportion unfed parous | Proportion blooded | Proportion gravid | Estimated mean number exposed to blood meal |
|-------------------|---------------------|-----------------------------|-------------------------|-------------------|-----------------|------------------------------------------|
| AGT               | 4.60 (1.96)         | 0.12                        | –                       | 0.311             | 0.57            | 4.05                                     |
| BOX               | 0.92 (0.33)         | 0.13                        | 0.13                    | 0.105             | 0.63            | 0.79                                     |
| CDC               | 0.15 (0.07)         | –                           | –                       | 0.5               | 0.5             | 0.15                                     |
| LIT               | 1.19 (0.64)         | 0.64                        | 0.3                     | 0.03              | 0.03            | 0.43                                     |
| ET                | 2.11 (0.84)         | 0.13                        | 0.17                    | 0.46              | 0.24            | 1.84                                     |
| PSC               | 3.05 (0.59)         | 0.16                        | 0.046                   | 0.66              | 0.13            | 2.55                                     |

AGT, *Anopheles gravid* trap; BOX, Box gravid trap; CDC, CDC gravid trap; LIT, light trap; ET, Exit trap and PSC, Pyrethrum spray catches.

*Estimates per collection night in the case of AGT, BOX, CDC, LIT, ET and per room for PSC.

### Table 3. Infection prevalence of *W. bancrofti* and *Plasmodium* spp. in mosquito pools

| Region       | Total number of mosquitoes screened | Estimated prevalence *W. bancrofti* DNA (%) (95% CI) | Estimated prevalence *Plasmodium* spp. DNA (%) (95% CI) | Trap type       |
|--------------|------------------------------------|------------------------------------------------------|--------------------------------------------------------|---------------|
| Western      | 382                                | 0.80 (0.155–2.30)                                     | 2.15 (0.867–4.28)                                      | IRC, BOX, AGT (Plas) |
| Northern     | 516                                | 3.01 (1.60–5.05)                                      | 3.27 (1.78–5.40)                                       | IRC, BOX, AGT (Plas) |

*The table above shows the estimated prevalence of *W. bancrofti* and *Plasmodium* spp. DNA in the mosquitoes collected using PoolScreen 2.0.

However, there is a challenge identifying efficient vector collection methods for anopheline mosquitoes, the primary vectors in sub-Saharan Africa for both LF and malaria. Using gravid traps increases the chance of catching infected mosquitoes as gravid mosquitoes would have taken at least one blood meal (Irish *et al.*, 2013). CDC gravid traps, purposely designed for *Culex* mosquitoes, are used routinely for the surveillance of diseases such as dengue fever and lymphatic filariasis transmitted by *Culex* mosquitoes (L’Ambert *et al.*, 2012; Hapairai *et al.*, 2015). However, an equivalent for anophelines is not widely used for monitoring, and this has been a challenge for surveying these populations. This study reports on the first evaluation of an *Anopheles* gravid trap for monitoring LF and malaria in vectors in Ghana.

For this study, trapping efficiency was evaluated by comparing the mean number of *Anopheles* per trap night. Amongst the gravid traps used, the AGT performed better than the Box gravid trap (BOX) and CDC-gravid trap (CDC), even though all three were baited with water from larval habitats. AGT had the highest proportion of *An. gambiae s.l.* exposed to a blood meal as well as the highest mean number of *An. gambiae s.l.* compared with the other collection methods in the Western region. Its efficiency at trapping exposed mosquitoes was approximately 1.6 and 2.2 times better than PSC and ET, respectively. Amongst the outdoor collection methods, it was 5.1, 27 and 9.4 times better than BOX, CDC and LIT. The improved performance of AGT could be due to a bigger fan, which provided a suction effect over the entire water surface. Whereas, the suction effect for the BOX and CDC were only strong at the opening to the collection chambers but the effect was less towards the periphery. AGT resulted in a higher catch than Exit trap (ET), CDC-LIT and pyrethrum spray collection (PSC), when standardized per location per night. Similar observations were made in the testing of AGT in Kenya (Lindh *et al.*, 2016). The Kenyan study evaluated the trapping efficiency of the OviART gravid trap designed to collect gravid *Anopheles*. The OviART gravid trap, which is similar to the AGT used in this study, collected 2.3 times the number of *An. gambiae s.l.* compared with BOX. In our study, the AGT...
also showed good trapping efficiency for An. melas, even though this species prefers to breed in high salinity water compared with other anophelines. AGT also caught the highest mean number of Anopheles that had previously taken a blood meal. Similarly, Lindh et al. (2016) observed a higher proportion (90%) of gravid mosquitoes in the OviART trap. The main difference in the AGT and OviART trap is the oviposition tray. The AGT had a 6 L rectangular basin unlike the OviART which had 8 L circular basin in which water was kept serving as an attractant for gravid mosquito. The rectangular basin of the AGT provides a larger surface area for oviposition, compared with the circular basin of the OviART. However, it is not clear whether the aforementioned variations, affects the performance of these traps.

Some of the limitations to the use of the AGT are due to bulkiness and the use of a 12 V car battery which limits portability. There is no protective shield on the traps and the basins get flooded during rains wetting the collection chambers and trapped mosquitoes. Rains can also damage the fan and the battery. As such, improved designs aimed at protecting the components of the trap while also improving portability will make it more useful in the field.

The high numbers and the different species caught in the AGT show that, not only is it efficient for sampling LF and malaria vectors, it can also be employed in sampling other mosquito vectors including Culex which is implicated in LF and arbovirus transmission elsewhere (Mak, 2007; Lutomiah et al., 2011; Jones et al., 2012). Information such as vector abundance and diversity within a locality, can help inform local health authorities on vector distribution and implication in the transmission which can be the basis for deploying control measures (Ciota and Kramer, 2013; Oduola et al., 2013). Further, parasite positivity in mosquitoes collected with these traps indicates the presence of infection in the community, providing information that may require intensified efforts to manage and control vector-borne diseases (Kouassi et al., 2015).

None of the head/thorax pools of mosquitoes were positive for W. bancrofti and Plasmodium spp. in the Western region, suggesting that these mosquitoes were not carrying any infective stages. However, a small number of pools were positive from the Northern region. The mosquitoes that were positive for W. bancrofti DNA were An. coluzzii and An. melas, the primary vectors of W. bancrofti in these areas (de Souza et al., 2010). The number of samples analysed was few, however, they illustrate the utility of detecting parasite DNA in mosquitoes, even when infection prevalence is very low in the community. Detection of parasite DNA confirms the presence of infection in humans and indicate ongoing transmission to mosquitoes. Furthermore, the positive pools obtained from the traps, supports the evidence that these methods are useful for sampling epidemiologically relevant mosquitoes (ie. mosquitoes that were exposed to at least a blood meal). Hence, they can be employed in monitoring vector populations which can provide valuable information to support the decision to stop MDA.

However, comparison of the differences in infection prevalence in mosquitoes between collection methods of Anopheles species and locations were not performed. This is a limitation as these information are relevant and can inform vector monitoring campaigns.

In conclusion, AGT was a very efficient collection method compared with the other traps in both study regions, but particularly in the Western region where few mosquitoes were found resting indoors. We found that, on average, AGT collected over 2 times as much as blood exposed Anopheles compared with the indoor methods and 5–27 times compared with the other gravid traps. The collection of indoor resting mosquitoes, either by PSC or mechanical aspiration is efficient in areas with high numbers of indoor resting anophelines such as in the Northern region since many rooms can be screened for indoor resting mosquitoes by one team of collectors. While the AGT showed efficiency in trapping mosquitoes, there are limitations to the number of traps that can be set and rotated by a team because of the heavy car battery, which needs to be recharged every few days.

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Conflict of interest. None.

Ethical standards. Ethical approval for the study was obtained from the IRB of the Noguchi Memorial Institute for Medical Research (approval number: 100/15-16) and the Liverpool School of Tropical Medicine’s Ethical Committee (approval number: M11-17). Consent was obtained from members of households before undertaking indoor collections. Written informed consent was obtained before testing individuals for W. bancrofti parasites.

References
Agbolade OM and Akinboye DO (2005) Detection of microfilariae with counting chamber technique in some Nigerian rural communities. African Journal of Biotechnology 4(1), 367–370.
Amazu H, Wilson MD and Boakye DA (2010) Studies of Anopheles gambiae s.l (Diptera: Culicidae) exhibiting different vectorial capacities in lymphatic filariasis transmission in the Comoros district, Ghana. Parasites & Vectors 3, 85–88.
Boakye DA, Wilson MD, Appawu MA and Gaypong J (2004) Vector competence for Wuchereria bancrofti, of the Anopheles populations in the Bongo district of Ghana. Annals of Tropical Medicine and Parasitology 98, 501–508.
Brady M (2014) Seventh meeting of the global alliance to eliminate lymphatic filariasis: reaching the vision by scaling up, scaling down, and reaching out. Parasites and Vectors 7(1), 46.
Bryan JH, McMahon P and Barnes A (1990) Factors affecting transmission of Wuchereria bancrofti by anopheline mosquitoes. 3. Uptake and damage to ingested microfilariae by Anopheles gambiae, An. arabiensis, An. merus and An. funestus in east Africa. Transactions of the Royal Society of Tropical Medicine and Hygiene 84(2), 265–268.
Budge PJ, Dorkenoo AM, Sodahlon YK, Fasuyi OB and Mathieu E (2014) Ongoing surveillance for lymphatic filariasis in Togo: assessment of alternative and nationwide reassessment of transmission status. The American Journal of Tropical Medicine and Hygiene 90(1), 89–95.
Burkot T and Ichimori K (2002) The PacELF programme: will mass drug administration be enough? Trends in Parasitology 18(3), 109–115.
Chanteau S, Luquiaux P, Failloux AB and Williams SA (1994) Detection of Wuchereria bancrofti larvae in pools of mosquitoes by the polymerase chain reaction. Transactions of the Royal Society of Tropical Medicine and Hygiene 88(6), 65–66.
Ciota AT and Kramer LD (2013) Vector–virus interactions and transmission dynamics of West Nile virus. Viruses 5(12), 3021–3047.
Derua YA, Rumisha SF, Batengana BM, Max DA, Stanley G, Kisinza WN and Mboera LE (2017) Lymphatic filariasis transmission on Mafia Islands, Tanzania: evidence from xenomonitoring in mosquito vectors. PLoS Neglected Tropical Diseases 11(10), e0005938.
de Souza D, Kelly-Hope L, Lawson B, Wilson M and Boakye D (2010) Environmental factors associated with the distribution of Anopheles gambiae s in Ghana; an important vector of lymphatic filariasis and malaria. PLoS ONE 5(3), e9927.
de Souza DK, Koudou B, Kelly-Hope LA, Wilson MD, Bockarie MJ and Boakye DA (2012) Diversity and transmission competence in lymphatic
filariasis vectors in West Africa, and the implications for accelerated elimination of Anopheles-transmitted filariasis. Parasites & Vectors 5, 25.

de Souza DK, Sesay S, Moore MG, Ansumana R, Narh CA, Kollie K, Rebollo MP, Koudou BG, Korama JB, Bolay FK, Bockarie MJ and Boakye DA (2014) No evidence for lymphatic filariasis transmission in big cities affected by conflict related rural-urban migration in Sierra Leone and Lymphatic Tropical Diseases 8(2), e2700.

Detinova TS (1962) Age grading methods in Diptera of medical importance with special reference to some vectors of malaria. World Health Organization (WHO) (Geneva, Switzerland) Monograph Series 47, 1–216.

Dugassa S, Lindh JM, Oyieke F, Mukabana WR, Lindsay SW and Fillinger U (2013) Development of a gravid trap for collecting live malaria vectors Anopheles gambiae s.l. PLoS ONE 8(7), e69948.

Eidman DF, Betson MA, Aikawa Y, Davidson J, Xiao H, Niedalski J, Xu Y, Stevenson JC, Bugoro H, Aparaimo A, Reuben H, Cooper R, Burkt RT, Russell TL, Collins FH and Lobo NF (2017) Fast and robust single PCR for Plasmodium sporozoite detection in mosquitoes using the cytochrome oxidase I gene. Malaria Journal 16(1), 230.

Fachinelli L, Koenaardt CJM, Fanello C, Kijchalao U, Valerio L, Jones JW, Lom villages affected by conflict related rural-urban migration in Sierra Leone. Journal of Tropical Medicine and Hygiene 78(6), 904–909.

Fanello C, Santolamazza F and Della Torre A (2002) Simultaneous identification of species and molecular forms of the Anopheles gambiae complex by PCR-RFLP. Medical and Veterinary Entomology 16(4), 461–464.

Farid HA, Morsy ZS, Helmy H, Ramzy RM, El Setouhy M and Weil GJ (2007) A critical appraisal of molecular xenomonitoring as a tool for assessing progress toward elimination of Lymphatic Filariasis. The American Journal of Tropical Medicine and Hygiene 77(4), 593–600.

Gillies MT and Coetzee MA (1987). Supplement to the Anopheline of Africa South of the Sahara. Johannesburg: South African Institute for Medical Research, pp. 1–143.

Hapairai LK, Pitchart C, Naseri T, Silva U, Tesimale L, Pemita P, Bossin HC, Burkt RT, Ritchie SA, Graves PM, Melrose W and Joseph H (2015) Evaluation of traps and lures for mosquito vectors and xenomonitoring of Wuchereria bancrofti infection in a high prevalence Samoan Village. Parasites and Vectors 8, 287.

Ichimori K, King JD, Engels D, Yajima A, Mikhailov A, Lammie P and Ottesen EA (2014) Global programme to eliminate lymphatic filariasis: the processes underlying programme success. Plos Neglected Tropical Diseases 8(12), e3328.

Irish SR, Moore SJ, Derua YA, Bruce J and Cameron MM (2013) Development of a gravid trap for collecting live malaria vectors in vector and human populations from urban communities in Oyo State, Southwestern Nigeria. Parasitology Research 112(10), 3433–3439.

Ochora PN and de Souza DK (2016) Prospects, drawbacks and future needs of xenomonitoring for the endpoint evaluation of lymphatic filariasis elimination programs in Africa. Transactions of the Royal Society of Tropical Medicine and Hygiene 110(2), 90–97.

Ottesen EA (2006) Lymphatic filariasis: treatment, control and elimination. Advances in Parasitology 61, 395–441.

Pederson ME (2008) Vectors of lymphatic filariasis in Eastern and Southern Africa. In Simonsen EP, Malecela NM, Michael E and Mackenzie DC (eds), Lymphatic Filariasis Research and Control in Eastern and Southern Africa. Denmark: DFL-Centre for Health Research and Development, Kailow Graphics A/S, pp. 78–110.

Springer YP, Hoekman D, Johnson PT, Duffy PA, Hufn RA, Barnett DT and Beard CB (2016) Tick-, mosquito-, and rodent-borne parasite sampling designs for the National Ecological Observatory Network. Eosphere (Washington, DC) 7(5), e01271.

Stolk WA, Swanisathan S, Oortmanssen GJV, Das PK and Habbema JDF (2003) Prospects for elimination of bancroftian filariasis by mass drug treatment in Pondicherry, India: a simulation study. The Journal of Infectious Diseases 188, 1371–1381.

Tabuer RN, Awono-Ambene P, Etang J, Atangana J, Antonio-Nkondjio C, Toto JC, Patchoke S, Leke RG, Fondjo E, Mnzava AP, Knox TB, Tougordi A, Donnelly MJ and Bigoga JD (2017) Role of Anopheles (Cellia) Rufipes (Gough, 1910) and other local anophelines in human malaria transmission in the northern savannah of Cameroon: a cross-sectional survey. Parasites & Vectors 10(1), 22.

Tuno N, Kjaerandsen J, Badu K and Kruppa T (2010) Blood-feeding behavior of Anopheles gambiae and Anopheles melas in Ghana, western Africa. Journal of Medical Entomology 47:28–31.

Ugahsi J, Bekard HE, Coulibly M, Adahie-Gomez D, Gyapong J, Appawu M, Wilson MD and Boakye DA (2012) Massonina africana and Massonaria uniformis are vectors in the transmission of Wuchereria bancrofti lymphatic filariasis in Ghana. Parasites & Vectors 5(1), 89.

Vazquez-Prokopec GM, Spillmann C, Zaidenberg M, Kitron U and Gürtler RE (2009) Cost-effectiveness of Chagas disease vector control strategies in northwestern Argentina. PLoS Neglected Tropical Diseases 3(1), e363.

Wanjiru S, Amvongo-Adja N, Koudou B, Njouenou DJ, Ndongo PW, Kengne-Ouafa JA, Datchou-Poutchou FR, Foyenou BA, Tayong DB, Femba JE, Supphar P, Fisher PE, Leponce M and Rockaert M (2015) Cross-reactivity of filarias ICT cards in areas of contrasting endemicity of Loa loa and Mansomina perstans in Cameroon: implications for shrinking of the lymphatic filariasis map in the Central African Region. PLoS Neglected Tropical Diseases 9(11), e0004184.

Williams GM and Gingrich JB (2007) Comparison of light traps, gravid traps, and resting boxes for West Nile virus surveillance. Journal of Vector Ecology 32(2), 285–291.
World Health Organisation (2011) Monitoring and Epidemiological Assessment of Mass Drug Administration in the Global Programme to Eliminate Lymphatic Filariasis: A Manual for National Elimination Programmes. (WHO/HTM/NTD/PCT/2011.4). Geneva, Switzerland: World Health Organization.

World Health Organisation (2015) Global programme to eliminate lymphatic filariasis: progress report, 2014. Weekly Epidemiological Record 90, 489–504.

World Health Organisation (2016) Global Programme to Eliminate Lymphatic Filariasis: Progress Report, 2015. Geneva, Switzerland: World Health Organization.

World Health Organisation (2017) Togo: First Country in sub-Saharan Africa to Eliminate Lymphatic Filariasis. Brazzaville, Geneva: World Health Organization.