

Spatial patterns of black fly (*Simulium damnosum* s.l.) dispersal in Ghana and Benin and their implications for onchocerciasis surveillance

Irene Kyomuhangi^{1,2†}, Manuela Carnaghi^{3†}, Pelagie Boko-Collins⁴, Bernix B. Soglongbe^{5,6}, Zinsou C. Koukpo⁷, Kenneth B. Otabil⁸, Francis B. D. Veriegh⁸, Claudio Fronterre^{1,9}, Maria-Gloria Basáñez¹⁰, Robert A. Cheke^{3,10}, Frances M. Hawkes^{*3}

¹ Centre for Health Informatics, Computing and Statistics, Lancaster University, UK

² Bennett Institute for Applied Data Science, University of Oxford, UK

³ Natural Resources Institute, University of Greenwich, UK

⁴ Sightsavers, Cotonou, Benin

⁵ Centre Suisse de Recherche Scientifique en Côte d'Ivoire, Côte d'Ivoire

⁶ University of Abomey-Calavi, Benin

⁷ Centre de Recherche Entomologique de Cotonou, Ministry of Health, Benin

⁸ Consortium for Neglected Tropical Diseases and One Health, University of Energy and Natural Resources, Sunyani, Ghana

⁹ Department of Applied Health Sciences, University of Birmingham, UK

¹⁰ MRC Centre for Global Infectious Disease Analysis, Department of Infectious Disease Epidemiology, School of Public Health, Imperial College London, UK

[†] Irene Kyomuhangi and Manuela Carnaghi contributed equally to this work.

*Correspondence: f.m.hawkes@greenwich.ac.uk

- 23 IK: irene.kyomuhangi@phc.ox.ac.uk
- 24 MC: manuela.carnaghi@gmail.com
- 25 PB-K: pboko@sightsavers.org
- 26 BBS: bertillesoglongbe@gmail.com
- 27 ZCK: zkoukpo@yahoo.fr
- 28 KBO: kenneth.otabil@uenr.edu.gh
- 29 FBDV: francis.veriegh@uenr.edu.gh
- 30 CF: c.fronterre@bham.ac.uk
- 31 M-GB: m.basanez@imperial.ac.uk
- 32 RAC: r.a.cheke@greenwich.ac.uk
- 33 FMH: f.m.hawkes@greenwich.ac.uk

Abstract

Background: Entomological surveillance for onchocerciasis is closely linked to riverine environments where black fly vectors breed. Relying solely on this xenomonitoring method may underestimate exposure risk in communities further away, as black flies can disperse over hundreds of kilometres. As endemic countries aim for elimination, understanding spatial heterogeneities in entomological indices is crucial.

Methods: We therefore conducted spatially explicit entomological surveys around two historical breeding sites: Bétérou in Benin and Asubende in Ghana, during the 2023 and 2024 wet seasons. Using a radial sampling design, 40 settlements within 25 km around each site were selected. Black flies were collected via Human Landing Catches at both breeding sites and settlements, and one Esperanza Window Trap at each settlement. Black flies were identified morphologically; biting rates were calculated relative to sampling effort; parity assessed by dissection; and parasite presence determined through O-150 PCR pool-screening and dissection. Generalised Additive Models described trends in entomological indices over distance.

Results: A total of 76,050 black flies was collected over 656 sampling days, primarily in Bétérou. In Bétérou, black fly counts and biting rates generally declined with increasing distance from the breeding site, though notable spatial variation was observed. Positive O-150 PCR pools were found at 31 of 41 sites, with infective flies detected up to 19 km from the breeding site. Bétérou exceeded the WHO infectivity prevalence threshold for interrupted transmission in 2023 (0.11%) and 2024 (0.08%). Parous rates remained relatively constant across distance. In Asubende, similar trends were observed, but low catches limited trend analysis, and no infective flies were detected via PCR.

Conclusions: These findings demonstrate that black fly dispersal and infectivity capable of sustaining transmission extend beyond breeding sites and first-line villages. This underscores

59 the importance of broader, spatially extensive vector sampling to better evaluate transmission
60 patterns and inform elimination efforts. To gain deeper understanding of the underlying
61 spatial dynamics beyond descriptive trend analysis, comprehensive spatial-temporal
62 modelling is required.

63 **Keywords:** Black fly dispersal, *Simulium damnosum* sensu lato, Onchocerciasis,
64 Entomological surveillance, Xenomonitoring, Elimination of transmission.

Introduction

Onchocerciasis, also known as river blindness, is a debilitating parasitic disease caused by infection with the filarial nematode *Onchocerca volvulus* and is transmitted by haematophagous black flies (*Simulium* spp.). The infection can lead to acute and chronic dermatological conditions [1], visual impairment and blindness [2] and increase mortality [3]. A dose-response relationship between microfilarial load in childhood and the probability of developing epilepsy later in life has been documented [4, 5], contributing to the epidemiological evidence of onchocerciasis-associated epilepsy. In 2021, an estimated 19.6 million people were infected by *O. volvulus* and onchocerciasis accounted for 1.26 million disability-adjusted life years (DALYs) [6], mostly in sub-Saharan Africa. The principal African vectors are members of the *Simulium damnosum* species complex, the cytospecies and cytoforms of which differ in habitat preferences, vector competence and vectorial capacity [7, 8], with implications for local transmission dynamics and surveillance design [9]. Onchocerciasis transmission is associated with riverine environments because larval development of black flies requires fast-flowing and well-oxygenated water [10]. However, adult females range away from natal rivers in search of blood-meal hosts, thus dispersing into surrounding environments and extending infection exposure risk beyond focal breeding areas.

Although black fly dispersal, driven by host-seeking appetitive flight, is a fundamental determinant of onchocerciasis epidemiology, it remains poorly understood due to literature being scarce, and at times contradictory [11]. During the World Health Organization (WHO) Onchocerciasis Control Programme in West Africa (OCP, 1974–2002), reinvading flies were found to travel more than 500 km from their breeding sites to reach rivers previously treated with larvicide [12]. Earlier studies closer to breeding sites showed that biting females were found up to 40–55 km away from their breeding sites [13–18]. Le Berre [15] expressly reported biting rates at a range of distances from a breeding site in Côte

d'Ivoire, recording 1800 bites per person per day at the riverbank, dropping to 16 bites per person at 41 km away, suggesting a general decline in biting density with increasing distances from breeding sites. Other results differ, including from within the same study area [19, 20]. In observations from Cameroon that were not statistically analysed, Duke (1975) found between 2 and 3 times more biting flies 3 km away compared to at the river itself, but this varied according to month [17].

Other vector characteristics that influence transmission, such as parity (an indicator of a fly's physiological age) and infectivity status, may also differ spatially; however, evidence of this is mixed. Classical studies in savannah biomes report decreasing parous rates with greater distance from breeding sites [15, 17, 18], consistent with a higher concentration of older females, which are therefore more likely to be infective, near rivers. Walsh *et al.* estimated a decrease in parity rate of around 1.11% with each additional kilometre up to 10 km from the breeding site, implying greater short-range dispersal distances among nulliparous flies [21]. In contrast, only one out of three savannah study sites, where *Simulium sirbanum* was the main vector, followed this pattern [22] and most long-distance migrants are reported to be parous [12].

With respect to black fly infectivity with *O. volvulus*, active transmission has been found from several to hundreds of kilometres away from breeding sites [21, 23]. At a site in Ghana, communities were sampled within a 20-km radius from the breeding site but transmission was confined to within 10 km of the river [24], whereas in Cameroon, annual transmission potential (ATP, the number of infective L3 larvae/person/year) increased with distance from the breeding sites, reaching as high as 813 at a village located 32 km from the nearest breeding site [25]. Many long-distance migrant vectors in the OCP harboured infective larvae [12].

Routine entomological collections have historically been concentrated at first-line villages, i.e. those lying on the riverside or, most commonly, at critical black fly breeding hotspots [21]. The discrepancies in the literature suggest that black fly biting, parous and infection/infectivity rates across spatial gradients from riverine environments are complex, and their quantitative impact on onchocerciasis exposure risk at and away from breeding sites may differ. Entomological sampling limited to riversides may thus over- or under-estimate transmission risk elsewhere. Understanding how biting rates and infectivity levels change at different distances from breeding sites could, therefore, be central to designing robust entomological surveillance and intervention strategies. This knowledge is essential for guiding targeted interventions, understanding variations in their impact on (proximate) communities, calibrating transmission dynamics models used to project elimination trajectories [26], and delineating transmission zones that are ecologically and epidemiologically meaningful [27, 28]. To evaluate progress towards interruption (elimination) of transmission, the WHO has proposed, for entomological surveillance, a target infectivity prevalence of less than 0.05% (at the upper 95% confidence bound) in black fly vector samples of at least 6,000 flies collected throughout a transmission zone and tested for the presence of *O. volvulus* DNA in their heads [29]. This threshold assumes that the proportion of parous flies in the sample is 0.5 and requires that all flies be negative to demonstrate <0.05% infectivity prevalence at the upper 95% confidence limit [29]. A perfect (100%) sensitivity and specificity of the diagnostic tool for xenomonitoring is also implied.

This study aimed to quantify how vector biting, parity, and different infectivity indices varied with distance from two breeding sites: one in Bétérrou in Benin, and the other in Asubende in Ghana. Both sites had served as black fly collection points during the OCP and thus have extensive associated historical datasets. Building on this context, and in contrast to standard entomological surveillance approaches that focus primarily on breeding

sites and/or nearby first-line villages, we implemented a spatially explicit radial sampling design covering 40 settlements within a 25-km radius of each breeding site. This design allows a direct, empirical characterisation of how crucial entomological indices change across an extended distance gradient under operationally realistic surveillance conditions.

Methods

Study areas

The study was conducted in Bétérou, Benin, and Asubende, Ghana in the wet seasons of 2023 and 2024. A breeding site was identified along the Ouémé River (9° 11.89' N, 2° 16.02' E) in Bétérou and the Pru River (8° 1.14' N, 0° 58.88' W) in Ghana. Both sites support black fly populations previously identified as being predominantly *S. damnosum* sensu stricto (s. s.) and *S. sirbanum*, with some Beffa form of *S. soubrense* at Bétérou [30-33] and are endemic for onchocerciasis [34]. Bétérou and Asubende were selected as examples where there was a single major black fly breeding area on the main river and its surrounding catchment, with no other major river systems supporting substantial black fly populations within approximately 50 km, and due to their history as vector monitoring sites for the OCP, providing accessible historical entomological and onchocerciasis data. The Benin national Neglected Tropical Diseases programme conducted a breeding site assessment exercise in 2018 and found no salient black fly breeding sites near to Bétérou [P. Boko-Collins, pers. comm.]). Likewise, after careful prospection in the Pru district in which Asubende lies, Chikezie *et al.* (2024) found no other riverine habitat able to support black fly larvae production other than along the rapids within 2 km of Asubende [5]. Both sites underwent vector control (by aerial larviciding) during the OCP from the late 1980s as part of the southern extension [29], ending in 2002 in Benin and 2001 in Ghana. In Asubende, vector control began in 1986 and was interrupted in 1987–1989 for the first community ivermectin trial [35, 36]. Annual ivermectin

mass drug administration (MDA) started in 1996 in Bétérrou and in 1987 in Asubende. After the closure of the OCP, the Upper Ouémé (Benin) and Pru (Ghana) river basins were designated as Special Intervention Zones (SIZ); vector control continued until 2007 with biannual MDA (2003–2007) in the Upper Ouémé, but not in the Pru [37]. From 2008 onwards, Benin switched to annual MDA nationwide, whereas biannual treatment has been implemented in Asubende since 2010 [38]; both areas continue to receive MDA according to these schedules.

In addition to the collection of adult *S. damnosum* s.l. adjacent to the riverine breeding sites at Bétérrou and Asubende, 40 additional settlements located within a 25-km radius of each of the two breeding sites were selected for the study. This resulted in a total of 41 sites in each study area from which adult blackflies were collected.

Sampling

Sampling was carried out over an eight-week period during the wet seasons of 2023 and 2024. In 2023, sampling took place in Benin from August to September and in Ghana from late August to early October. In 2024, it was conducted in Benin from late July to early September and in Ghana from late September to early November.

In both countries, adult black flies were sampled from a collection point located on the riverbank close to the breeding site and from a collection site in each of 40 settlements within a 25-km radius of the breeding site. Settlements were identified through the Geo-Referenced Infrastructure and Demographic Data for Development (GRID3-SE) settlement extent dataset, and their population estimates derived from the WorldPop project [38, 39]. Only settlements with populations greater than 100 individuals were included. The selection process employed a lattice plus close-pairs design to capture small-scale spatial variation while achieving comprehensive spatial coverage. The process began by randomly selecting

30 settlements within a 25-km radius of the breeding site, ensuring that they were at least 5 km apart. From these, a random subset of 10 settlements was selected, and for each settlement, the nearest neighbouring settlement was also included, resulting in a total of 40 settlements sampled. Initial collection sites in the sampled settlements were determined by the centroid of the settlement. For instances where the proposed collection site was found to be unsuitable for sampling during initial site visits, the closest suitable position to the initial collection site was selected. The most common reasons for modifying the collection site were because it was unacceptable to the community (e.g. on football pitches or sanctified ground), it was inaccessible (e.g. rocky outcrops or required crossing waterways), or if the collection process might disrupt day-to-day community activity and vice versa (e.g. at main thoroughfares or marketplaces).

Whenever feasible, sampling was carried out at the same sites across both years to ensure consistency. However, due to variations in accessibility between years (such as floods, political tensions, or land-use changes), one collection site in Benin was relocated in 2024 to a position 53 metres from its 2023 location. Additionally, 12 collection sites in Ghana were moved in 2024 to distances ranging from 52 to 1,356 metres from their respective 2023 positions (Figure 1).

Black flies were collected over an 8-week period using Human Landing Catches (HLC) and Esperanza Window Traps (EWT). At the breeding site, HLC collections only occurred, and took place once weekly throughout the entire eight-week period, resulting in eight trapping days in each study year.

At the 40 collection sites, both HLC and EWT were deployed using a balanced four-day cross-over study design at each location, whereby blackflies were sampled with one EWT and one HLC every day, and their positions were swapped each day to minimise any positional effects. This resulted in 320 total trapping days in each study year. Collections took

place over four consecutive days within a single week for each location. Five collection sites were visited each week, allowing all 40 sites to be sampled at some point during the eight weeks (Figure 2). Due to the wide geographical spread of the sites, these groupings were determined based on operational factors such as access and between-site travel time.

Black fly trapping methods

Host-seeking adult black flies were collected using the HLC method, which is considered to be the standard, following established protocols [40]. Briefly, a human collector sat with lower legs exposed, trapping any black flies that landed on them into 5 ml tubes. Sampling occurred hourly from 7:00 to 18:00 hrs, with two trained collectors alternating 1-hour periods between collection and rest, maintaining at least 50 m distance during rest. Collectors refrained from smoking, cooking, or using insect repellents to avoid influencing black fly behaviour. They recorded hourly weather conditions as ‘sunny,’ ‘cloudy,’ or ‘rainy’, based on predominant sky cover and noted significant wind events. During rain, collection was suspended. Black flies were individually placed in 5 ml tubes, wrapped in moist cotton pads, and stored in cool boxes shielded from direct sunlight to prevent desiccation. They were then transported to the laboratory station twice a day and either dissected before storing in 80% v/v ethanol or transferred directly to 80% v/v ethanol within 24 hours from collection.

During both years, EWTs were constructed according to the same specifications. The trap consisted of a 1 m² tarpaulin panel supported in either a wooden frame, which was used in Benin, or steel frame, which was used in Ghana, according to local fabrication options, positioned with its bottom edge approximately 15 cm above ground level. The tarpaulin was coated on both sides with TAD All Weather insect adhesive (Trécé, USA, previously branded as Tangle Foot and Tangle Trap) and baited with carbon dioxide generated from fermentation of a yeast-sugar mixture [41] and human odours. Human odour was presented via polyester

socks provided by one of the two HLC collectors, who wore them for 12 hours from 18:00 to 06:00 hrs the night before the collection day [42]. The tarpaulin was divided into three equal vertical portions arranged in a blue-black-blue pattern. The trap was set up prior to daily collection, with socks replaced daily and adhesives checked regularly for contamination, spot cleaned and replaced as needed. Carbon dioxide was produced fresh each morning and vented through a plastic tube attached to the frame, while socks were fixed to the top of the trap frame. During collection periods, black flies landing on the tarpaulin were collected at 12:00 hrs and 17:00 hrs and labelled accordingly. Collected black flies were carefully removed from the adhesive surfaces using forceps and kerosene as a solvent and stored in labelled vials containing 80% v/v ethanol for subsequent identification and analysis. When rain fell or when not in use, the trap was placed under a shelter to prevent wash-off or damage.

At each of the 40 collection sites, both HLC and EWT methods were used simultaneously, for four days per site, with the HLC collector and EWT spaced 20 to 40 metres apart and sheltered under trees or canopies. To reduce positional bias, the placement of the two methods was alternated daily.

Black fly species identification and dissection for parity status

For each site, a minimum of five flies captured per hour per day by HLC, or all flies captured during that period if fewer than five, were examined under a binocular microscope.

Additionally, all flies captured by EWT at the collection sites were also examined. These flies underwent initial morphological identification to confirm if they were *S. damnosum* s.l., based on criteria outlined by Freeman and De Meillon [43]. Following identification, the physiological age of black flies caught by HLC was determined through the presence or absence of ovarian relics, supported by other indicators of their reproductive status as either nulliparous or parous, as described by Lewis [44, 45]. Consequently, at least 55 black flies

per site per day captured by HLC, or all flies captured if fewer than 55, were identified and dissected to assess their parity status. Sub-samples from breeding sites were further classified to species or species group within *S. damnosum* s.l. using morphological characteristics detailed by Garms and Cheke [46]. Throughout the study, all collected black flies, regardless of whether they were dissected for parity status, were preserved in 80% v/v ethanol for subsequent parasite detection analyses.

Assessment of *O. volvulus* infections in black flies

Determination via dissection

A subset of the identified parous flies collected from the breeding sites in 2023 were dissected to detect any L1, L2 or L3 larvae indistinguishable from those of *O. volvulus* after staining with Mayer's Hemalum solution using established protocols [46].

Determination via O-150 PCR ELISA

The detection of *O. volvulus* DNA in the collected black flies was carried out using the O-150 PCR-ELISA technique, in accordance with established protocols [40, 47]. This method combines a polymerase chain reaction (PCR) assay targeting the O-150 repeat sequence specific to *O. volvulus* DNA with an enzyme-linked immunosorbent assay (ELISA) [48]. All flies collected by HLC were tested, with the exception of those collected at the breeding sites in 2023, of which a subset was dissected as described above. Briefly, black fly specimens preserved in 80% ethanol were pooled into groups of up to 100 flies, all originating from the same collections site. Heads of the flies were separated from bodies. Only pools of heads were tested and bodies kept at -80° C. For each pool of heads, the DNA was extracted using Dynabeads M280 Streptavidin solution. The PCR was conducted using the primers OV-150 F (5'-B-GATTYTTCCGRCGAANARCGC-3') and OV-150 R (5'-B-

GCNRTRTAAATNTGNAAATTC-3') (where B=biotin; N=A, G, C, or T; Y=C or T; and R=A or G). as previously described [47]. Samples were classified as positive if their optical density (OD) values exceeded the mean plus three standard deviations of negative control wells. Positive and negative controls were included in each PCR-ELISA run.

Analysis

The entomological indices used for analysis in this study are defined in Table 1.

In this study, calculation of monthly infective biting rate (MIBR) and monthly transmission potential (MTP), which are key metrics in black fly entomological surveillance, required adaptation of the standard formula because black fly collections did not align neatly with calendar months (Additional file 1: Table S1). Conventionally, MIBR and MTP are calculated using the number of days in the calendar month as a multiplier. While this approach is appropriate when the objective is to estimate these indices as they occur within a specific calendar month, it also implies that the respective values from different months are not directly comparable, as they vary solely due to differences in month length. To facilitate comparability across sites, we therefore calculated MIBR and MTP using a fixed length of 31 days for a 'typical' month.

Analyses in this paper are largely descriptive, reporting trends in entomological indices as they relate to distance from breeding sites. Generalised Additive Models (GAMs) were used to describe the relationship between HLC counts, EWT counts, parous rates and infectivity indices (including daily biting rates (DBRs), infectivity prevalence and MIBR), versus distance from the breeding site. All analyses were conducted in the R programming language (version 4.5.1) [49]. Confidence intervals (CIs) around proportions are exact (Clopper-Pearson) 95% CIs [50]. Graphs were generated using the gg2plot [51] package in R, and maps were created using the leaflet package [52].

Results

The following results pertain to *S. damnosum* s.l., therefore in what follows, the term "black flies" is used generically to refer to the whole complex, without distinguishing among its cytospecies, unless otherwise stated.

During the study an overall total of 76,050 black flies was captured by both HLC and EWT over 656 sampling days (Table 2). Most black flies (95.1%) were collected in Bétérou, with fewer (4.9%) collected in Asubende. In Bétérou, overall black fly counts were stable between 2023 and 2024, while in Asubende, counts were higher in 2023 than in 2024. HLC counts consistently exceeded EWT at both sites, during both years. For example, in Bétérou, HLC counts were approximately 13 times higher than EWT counts in both years.

In Bétérou, HLC yielded black flies at all sites. However, for EWT, 5.0% of sites in 2023 and 12.5% in 2024 had no black fly catches (Additional file 1: Table S2 and Figure S1). In Asubende, a considerably higher proportion of sites had no black fly catches with HLC (34.1% in 2023 and 65.9% in 2024) and EWT (55.0% in 2023 and 87.5% in 2024) (Additional file 1: Table S2 and Figure S2). Despite the very low EWT counts of *S. damnosum* s.l. in the Asubende area, these traps did catch very high numbers (e.g. >1,600 from 5 sites during week 3 in 2024) of *Simulium adersi*.

Parous rates were consistently higher in Bétérou than in Asubende in both 2023 and 2024 (Table 2). At both sites, parous rates were also higher in 2024 compared to 2023.

Subsets of 545 (21.4%) adult black flies collected by HLC at the Bétérou breeding site and 83 (22.0%) at the Asubende breeding site in 2023 were examined morphologically to determine to which taxa within the *S. damnosum* complex they belonged. At Bétérou, 163 nulliparous flies and 375 parous flies (98.7%) were identified as *S. damnosum* s. s. / *S.*

sirbanum and five nulliparous and two parous flies (1.3%) were assigned to the Beffa form of *S. soubrense*. At Asubende all 83 flies were *S. damnosum* s. s. / *S. sirbanum*.

HLC counts at the Bétérou breeding site varied weekly over both years (Figures 3a and 3b). At the collection sites, HLC counts, and the subsequent DBRs, generally decreased with increasing distance from the breeding site (Figures 3c–3f). Similarly, EWT counts showed a general decline with distance (Figures 3g and 3h). Model outputs for the GAM-generated trend lines shown in Figure 3 are detailed in Additional file 1: Table S3.

Although black fly counts in Asubende were substantially lower than those in Bétérou, weekly fluctuations were also observed at the breeding site (Figures 4a and 4b). Due to the low numbers of black flies captured in Asubende, it was challenging to distinguish spatial trends in the black fly count data (Figures 4c–4h). Nevertheless, a qualitative examination of the data indicates similar spatial patterns to Bétérou, with a general decline in HLC counts and DBRs as distance from the breeding site increases. In what follows, additional analyses are presented for Bétérou only.

There was no discernable trend in the relationship between parous rates and distance from the breeding site in Bétérou, as parity rates appeared to be fairly constant over the distances investigated, both for 2023 and 2024 (Figures 5a and 5b). Overall, a large proportion of sites had *O. volvulus*-positive PCR pools, with 31 sites (75.6%) recording a positive pool in at least one of the two collection years. There was no discernible trend over distance for either infectivity prevalence or MIBR (Figures 5c – 5f). Figure 6 illustrates the locations where positive pools were identified. These positive pools were found at collection sites up to 19 km away from the breeding site. In 2023 and 2024, 20 and 19 sites, respectively, had positive pools, including 8 sites that were positive in both years. Ten sites remained negative across both sampling years, while 23 out of the 41 sites changed status from positive to negative, or *vice versa*, between 2023 and 2024. All PCR pools of flies

collected in Ghana were negative, however sample sizes were too small to be interpreted meaningfully.

Of 377 parous flies dissected from the 2023 HLC collections at the Bétérou breeding site (representing 72% of the total of 526 parous flies collected), 17 (4.5%) were infected with L1, L2 or L3 larvae indistinguishable from *O. volvulus* and 11 (2.9%) harboured infective (L3) larvae, 8 of which had L3s in the head. Since the proportion of parous flies at the Bétérou breeding site for 2023 was 0.685, this would be 8 infective flies in 550 biting flies, for an infectivity prevalence of 1.45% (95% CI = 0.63, 2.85), the latter result being higher than the range (0.00–0.70%) recorded by O-150 pool-screening for the same year, but within the range (0.00–5.68%) recorded in 2024 (Additional file 1: Tables S4 and S5). Using dissection data on L3s recovered from heads only, MTPs of 102 and 81 L3/person/month for August and September 2023, respectively, were estimated. If all L3 larvae found anywhere in the flies' bodies in September were included, the MTP would increase to 324 L3/person/month (there was no increase in August when all L3s found were in heads). At the Asubende breeding site, no L3 larvae were found in 83 parous flies dissected but one fly contained two L2 larvae (Additional file 1: Tables S6 and S7). However, the age structure of the black fly populations at Bétérou and Asubende is likely to have been different, with a preponderance of younger flies at the latter (64% nullipars) compared to the former (42% nullipars) in 2023.

Discussion

This study examined the spatial distribution of *S. damnosum* s.l. abundance, biting rates, parity, and infectivity across various locations within a 25-km radius surrounding two historically important breeding sites in West Africa, namely, Bétérou (capture point 1819 in the OCP database) in Benin and Asubende (capture point 1202) in Ghana. Abundance and

biting rates generally declined with increasing distance from the breeding sites, while parity rates and infectivity did not exhibit a clear or consistent gradient with distance (in Benin).

The general decrease in black fly abundance with increasing distance from the breeding sites is consistent with but also considerably extends a previous short report by Duke in Cameroon, who described similar trends, but only at study sites of a maximum of 3 km from breeding sites [17]. These trends were seen in both a savannah breeding site, and two forest breeding sites, although there was no statistical analysis of the data. Le Berre also observed a similar decline in forest areas up to 41 km from breeding sites [15]. However, the GAM results for Bétérou indicate that distance alone does not fully explain the variability observed in both HLC and EWT counts; thus, other factors are likely to play an important role in accounting for the spatial differences. Moreover, there are instances of very high vector abundances across the Bétérou study site that indicate considerable heterogeneity in biting exposure and require further investigation. Some of the unexplained variation in biting rates may be attributable to the suitability of the HLC locations for successful catches, as this is highly variable yet difficult to explain, e.g. some sites that are close to each other beside the same river can provide markedly different results (R. A. Cheke, unpublished observations in Asubende area during January–March 2011 and elsewhere in West Africa).

The GAMs presented in this study are intended as descriptive tools to summarise broad spatial patterns in black fly catches relative to distance from breeding sites, rather than to fully explain the processes governing black fly dispersal. In particular, the number of black flies emerging from the breeding site varies over time and is expected to influence observed counts at surrounding collection sites, but this temporal dependence is not explicitly represented in the models. Additionally, environmental factors such as rainfall, temperature, and wind speed or direction, which are known to affect black fly dispersal and activity [53], may vary across locations and were not incorporated in the analyses. Furthermore, while this

study included extensive spatial sampling of black flies, limitations in sampling duration and timing should be noted. Operational constraints permitted only once-weekly collections at the breeding site and four days of sampling per week at alternate collection sites over the 8-week period. These restrictions added complexity to the data and should be considered when interpreting the findings. The GAMs do not explicitly account for the structured sampling schedule, including the timing and frequency of collections across sites. Consequently, the observed distance-related patterns should be interpreted as empirical summaries conditional on the sampling design, and a more comprehensive spatial–temporal modelling framework would be required to jointly account for these underlying processes.

In a related study within the same project (Kyomuhangi *et al* 2026 [54]), interviews with participants from the Asubende study area revealed that in their experience, peak biting occurred from August to October (Additional file 1: Figure S4). Therefore, we aligned our black fly collection schedule to coincide with this period. However, community members’ anecdotal observations indicated that recent weather patterns, including unusually dry conditions during the collection period, may have contributed to low black fly counts (in comparison with those recorded in Benin). Although the Ghana Meteorological Agency reported generally mildly wetter than normal conditions around the nearby town of Prang in 2023, most of central and northern Ghana had experienced intense to severe drought in the months preceding sampling in 2024, with normal to mild drought throughout [55]. Due to the high number of zero catches in Asubende, identifying spatial trends was difficult, highlighting the challenges of analysing black fly dispersal in areas with low vector densities and changing weather patterns.

In Bétérrou, parous rates remained relatively constant across different distances, and O-150 positive PCR pools were detected throughout the study area and up to 19 km from the breeding site. This indicates that older, potentially infective female black flies can both

disperse and continue to bite some distance from the breeding site, thereby contributing to transmission beyond first-line villages, as indicated by the MIBR vs distance relationship. Parous rates of savannah black fly species have previously been reported to decline over distances of 3 km [17], 10 km [18] and up to 41 km [15] from breeding sites. Our findings call into question the ubiquity of this trend, alongside results from Séchan [7], who found the pattern reversed between study years. The long-distance migratory behaviour of black flies also has a bearing on interpretation of parous and vector infective prevalence over distance. Studies on reinvasion by black flies into areas that had undergone vector control during the OCP and summarised in Cheke *et al.* [11] concluded that *S. damnosum* s. s. and *S. sirbanum* could travel up to 500 km. Such migrations were typically observed as movements from dry season quarters in the southwest to northeast in the wet season, and back again from northeast to southwest at the end of the dry season, reflecting seasonal wind-assisted movements associated with the Intertropical Convergence Zone. Migrants included a high proportion of infective flies and, moreover, nulliparous flies were never found more than 25 km from breeding sites [56]. Sampling during the expected height of the wet season reduced the likelihood that our results would be confounded by migration but cannot exclude the possibility of some influence.

The presence of *O. volvulus* infective black flies at collection sites located further from the Bétérou breeding site, coupled with vector infectivity prevalence exceeding the WHO threshold of 0.05% [29], highlights an ongoing risk of transmission that may not be fully captured by standard entomological surveillance methods focused solely on breeding sites. None of the individual sites reached the sample size of 6,000 black flies required by WHO for the assessment of transmission interruption [29, 40]. However, taken as a whole, 30,613 flies were analysed by O-150 PCR ELISA in 2023 and 31,929 in 2024, demonstrating infectivity prevalences of 0.11% (95% CI = 0.07%, 0.15%) and 0.08% (0.05%, 0.11%),

respectively, indicating ongoing transmission. Furthermore, PCR positive sites were widely dispersed across the whole study area and the majority of these changed their status from positive to negative, or *vice versa*, between 2023 and 2024, indicating the distribution of infective blackflies is widespread and high, as well as a spatially and temporally dynamic. These results support broadening surveillance efforts beyond breeding sites, particularly when conducting stop-MDA surveys and/or post-treatment and post-elimination surveillance. In Asubende, no PCR-positive pools were detected, but the sample sizes analysed were small. As one dissected black fly was found to contain L2 larvae, it is likely that transmission from humans to vectors is occurring, but additional sampling is recommended. This is because the total number of flies collected in Asubende over both years was considerably lower than the sample size proposed by the WHO guidelines for entomological surveillance [29, 40].

The conventional method for calculating MIBR and MTP was not appropriate in our study and may also be inappropriate when applied to different sampling strategies used in other surveillance and research studies. Our standardised formulation for these indices, using 31 days, yields an interpretable measure of transmission potential per ‘typical’ month and avoids artefactual differences driven purely by calendar structure. We suggest that this type of standardisation may be preferable when the primary goal is to compare MIBR and MTP across months or sampling periods, particularly in settings where collections are irregular or do not span full calendar months. Further, consultation with the research community is required to review how these key entomological indices are defined and to explore how different sampling methods can be accommodated, ensuring their relevance and comparability across diverse studies.

Conclusions

Our findings indicated that black fly dispersal and infectivity sufficient to maintain onchocerciasis transmission extend well beyond the first-line communities around the black fly breeding sites. This highlights the need for broader, spatially extensive vector sampling to effectively inform onchocerciasis control and elimination efforts, ensuring that transmission assessments are more representative of the heterogeneity in transmission risk for communities including those located further away from breeding sites.

Supplementary information

Additional file 1: Table S1. Months of black fly collection for the different sites in Benin (around the Bétérou breeding site) and Ghana (around the Asubende breeding site). **Table S2.** Numbers of sites with no black flies caught by Human Landing Catch (HLC) and Esperanza Window Trap (EWT) in Benin and Ghana. **Fig. S1.** Presence of (*Simulium damnosum* s.l.) black flies at different sites around the Bétérou breeding site, Benin. **Fig. S2.** Presence of (*Simulium damnosum* s.l.) black flies at different sites around the Asubende breeding site, Ghana. **Table S3.** Outputs of the Generalized Additive Models (GAMs) used to depict trend lines in Figures 3 and 5 of the Main Text, for sites around the Bétérou breeding site, Benin. **Fig. S4.** Percentage of participants indicating in which month black fly bites are worst. **Table S4.** Entomological indices for Bétérou, Benin 2023 by site number in order of distance from the breeding site (BS). **Table S5.** Entomological indices for Bétérou, Benin 2024 by site number in order of distance from the breeding site (BS). **Table S6.** Entomological indices for Asubende, Ghana 2023 by site number in order of distance from the breeding site (BS). **Table S7.** Entomological indices for Asubende, Ghana 2024 by site number in order of distance from the breeding site (BS).

Abbreviations

BS: Breeding site; DBR: Daily biting rate; EWT: Esperanza window trap; GAM: Generalised additive model; HLC: Human landing catch; MDA: Mass drug administration; MIBR: Monthly infective biting rate; MTP: Monthly transmission potential; PCR-ELISA: Polymerase chain reaction-enzyme linked immunosorbent assay; WHO: World Health Organization.

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Availability of data and materials

All raw data (excluding precise GPS coordinates) are available in the supplementary information, which are also available on Zenodo (DOI: <https://doi.org/10.5281/zenodo.18877634>). Exact spatial data are withheld due to ethical and privacy considerations. Data sufficient to reproduce all analyses, including derived distance measures, are provided in the public dataset.

Access to precise GPS coordinates may be granted upon reasonable request to the corresponding author (F.M.Hawkes@greenwich.ac.uk), subject to review of the proposed research purpose and completion of an appropriate data-sharing agreement. Where applicable, applicants may be required to provide evidence of institutional ethical approval. Data will be shared in accordance with relevant ethical and data protection requirements.

Authors' contributions

Conceptualisation: FMH and RAC. Methodology: FMH, RAC, CF, PB-C, KBO, FBDV. Investigation: MC, PB-C, BBS, ZCK, KBO, FBDV, FMH, RAC. Validation: IK. Software: IK, CF. Formal analysis: IK, CF, MC, PB-C, M-GB, FMH, RAC. Data curation: IK. Writing – original draft: IK, MC, FMH. Writing – review and editing: IK, MC, PB-C, BBS, ZCK, KBO, FBDV, CF, M-GB, RAC, FMH. Visualization: IK. Supervision: FMH. Project administration: FMH. Funding acquisition: FMH, RAC, CF, PB-C, KBO, M-GB.

Ethics approval and consent to participate

There were no human participants in this study. Human landing catches were approved by the Research Ethics Committee of the Institute of Applied Biomedical Sciences (CER-ISBA) in Benin (ref: N° 149 du 25/04/2023) and following Ghana Health Service best practice on

advice from the Committee for Human Research and Ethics of the University of Energy and Natural Resources in Ghana.

Competing interests

The authors declare that they have no competing interests.

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Figure captions

Fig. 1: The study sites in (a) Bétérou (Benin) and (b) Asubende (Ghana) for 2023 and 2024.

Red circles mark breeding sites; orange circles show collection sites consistent across both years; blue and pink circles denote sites where collection locations shifted by over 50 metres between 2023 and 2024. Insets for both (a) and (b) feature grey lines and shapes representing water bodies. Basemaps: CARTO Positron © OpenStreetMap contributors, © CARTO [57]

Fig. 2: Black fly collection schedule. This schedule involved weekly sampling over eight weeks. At the breeding site, black flies were collected once a week for eight weeks. At the 40 collection sites, black flies were collected over four consecutive days during one week only. Throughout the period, groups of five collection sites were visited each week, ensuring that all locations were sampled at least once during the study.

Fig. 3: Temporal and spatial patterns of black fly counts in Bétérou, Benin, for 2023 and 2024. The Human Landing Catch (HLC) counts at the breeding site across the 8 collection weeks are shown in (a) and (b). Figures (c) and (d) show the spatial trends in HLC counts across 40 collection sites, with the trend line generated using a Negative Binomial Generalised Additive Model (GAM). Figures (e) and (f) show spatial trends in estimated daily biting rates at all sites, with the trend line generated using a Gaussian GAM on log-transformed biting rates. Figures (g) and (h) show the spatial trends in Esperanza Window Trap (EWT) counts at the 40 sites, with the trend line generated using a Negative Binomial GAM.

Fig. 4: Temporal and spatial patterns of black fly counts in Asubende, Ghana, for 2023 and 2024. The Human Landing Catch (HLC) counts at the breeding site across the 8 collection weeks are shown in (a) and (b). Figures (c) and (d) show HLC counts across 40 collection sites. Figures (e) and (f) show estimated daily biting rates at all sites. Figures (g) and (h)

show Esperanza Window Trap (EWT) counts at the 40 sites. GAM-derived trend lines were unsuitable for (c) to (h).

Fig. 5: Spatial patterns for entomological indices in Bétérrou, Benin, for 2023 and 2024.

Trends in parous rates, generated using a binomial Generalised Additive Model (GAM), are shown in (a) and (b). Infectivity prevalences are shown in (c) and (d). Estimated monthly infective biting rates (MIBRs) are shown in (e) and (f); sites 3 and 34 had positive O-150 PCR pools, however, estimates of their infectivity rates are very uncertain due to the low number of pools or number of black flies (heads) in the pool, and therefore meaningful MIBRs could be not computed. GAM-derived trend lines were unsuitable for (c) to (f).

Fig. 6: Spatial distribution of positive and negative O-150 PCR pools in Bétérrou, Benin in 2023 and 2024. In the 2023 map, there are no PCR-derived infectivity results for the breeding site, which is indicated as a black circle (black flies were manually dissected rather analysed by molecular means). Dissection results revealed that infected and infective flies were present. Basemaps: CARTO Positron © OpenStreetMap contributors, © CARTO [57].

Tables

Table 1: Entomological indices, their definitions, calculations and units used in this study.

The term ‘black flies’ refers to *S. damnosum* s.l.

Index and Units	Definition and Calculation
HLC count	Number of black flies captured by Human Landing Catch (HLC)
EWT count	Number of black flies captured by Esperanza Window Trap (EWT)
Parous rate ^a	(number of parous black flies) / (number of black flies dissected for parity status)
Daily biting rate (DBR) ^a (bites/person/day)	(number of black flies collected by HLC) / (number of collection days)
Infectivity prevalence ^{a, b}	Proportion of infective flies (from O-150 PCR results) calculated using the R package PoolTestR [58], taking the Maximum Likelihood Estimate (MLE) of infectivity prevalence
Monthly infective biting rate (MIBR) ^a (infective bites/person/month)	$DBR \times \text{number of days in a month}^c \times (\text{infectivity prevalence})$
Monthly transmission potential (MTP) ^{a, b, c} (L3 (head)/person/month)	$DBR \times \text{number of days in a month}^c \times (\text{number of L3 larvae (head)}) / (\text{number of black flies dissected, i.e. both nulliparous and parous, for assessment of infectivity})$

^aDerived from black flies captured by HLC.

^bDerived from dissections of the heads of parous black flies captured by HLC.

^cFor consistency, the number of days in a month was standardised to 31 (see Methods).

787 Table 2: Human Landing Catch (HLC) and Esperanza Window Trap (EWT) counts, and
 788 parous rates for black flies collected in Bétérou, Benin and Asubende, Ghana in 2023 and
 789 2024.

		2023			2024		
		collection sites	breeding site	total	collection sites	breeding site	total
	Trapping days	320 days	8 days	328 days	320 days	8 days	328 days
<i>Bétérou</i>	HLC count	32,094	2,342	34,436	30,942	2,039	32,981
	EWT count	2,546	–	2,546	2,368	–	2,368
	Parous count	4,076	526	4,602	4,320	597	4,917
	Nulliparous count	3,112	242	3,354	2,852	168	3,020
	Parous rate (%) [95% CI]	56.7 [55.6, 57.9]	68.5 [65.1, 71.8]	57.8 [56.8, 58.9]	60.2 [59.1, 61.4]	78.0 [74.9, 80.9]	62.0 [60.9, 63.0]
<i>Asubende</i>	HLC count	1,754	954	2,708	177	377	554
	EWT count	439	–	439	18	–	18
	Parous count	481	237	718	83	208	291
	Nulliparous count	758	518	1276	91	168	259
	Parous rate (%) [95% CI]	38.8 [36.1, 41.6]	31.4 [28.1, 34.8]	36.0 [33.9, 38.2]	47.7 [40.1, 55.4]	55.3 [50.1, 60.4]	52.9 [48.6, 57.2]

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