DISSECT field toolkit for standardised black fly data collection

October 2025

REPUBLIC OF MOZAMBIQUE

MINISTRY OF HEALTH
NATIONAL DIRECTORATE OF PUBLIC HEALTH
Department of Disease Control and Prevention

Malawi Ministry of Health

GLOBAL INSTITUTE FOR DISEASE ELIMINATION

Sightsavers

Acknowledgements

The authors of this toolkit sincerely thank everyone involved with the DISSECT project. Special thanks to Maria Rebollo-Polo and Raman Velayudhan from WHO for providing a pre-release version of the Entomological manual of onchocerciasis elimination programmes. Gratitude is extended to Adrian Hopkins, Jeronimo Langa, Mike Osei-Atweneboana, Vincent Djohan, Dylo Pemba, Doris Harding, Joseph Kamgno, Frances Hawkes, and Monsuru Adeleke, who formed the project steering committee and contributed valuable inputs and improvements to this toolkit and wider project.

We are also deeply grateful to GLIDE for supporting and partnering with Sightsavers on this project. GLIDE contribution has enabled the development, testing and refinement of this field toolkit for standardised black fly data collection.

We extend our gratitude to the NTD coordinators and onchocerciasis managers in the Ministries of Health. Without whom the development and testing of this toolkit would not have been possible.

Thanks to the organisers and attendees of the COR-NTD meeting in October 2023, who reviewed the draft toolkit and gave feedback. Appreciation goes to the field teams in all four nations that directly tested the toolkit and provided iterative feedback on unexpected challenges. We acknowledge the efforts and patience of the administrative, procurement and logistics teams, who have supported all the vital preparations preceding any field work.

Finally, we extend our thanks to the resident communities where the toolkit was trialled for welcoming researchers, participating in the research and improving the quality of this toolkit.

List of Abbreviations

- NTDs: Neglected Tropical Diseases
- WHO: World Health Organization
- mf: Microfilariae
- L3: Third Stage Larvae
- MDA: Mass Drug Administration
- HLC: Human Landing Capture
- EWT: Esperanza Window Trap
- GPS: Global Positioning System
- pH: potential of Hydrogen
- CO_2: Carbon Dioxide
- VVC: Village Vector Collector
- CDD: Community Drug Distributor

Chapter 1 - Introduction

This document has been designed as a companion document to the World Health Organization's Entomology manual for onchocerciasis elimination programmes, to support the training of surveyors who will conduct capture of adult black flies. Here, the information given in the manual is summarized and simplified into easy to follow, step by step guidance.

This makes clear how countries should follow the current advice and best practice regarding conducting entomological breeding site assessments and transmission assessments for onchocerciasis. This toolkit will support Neglected Tropical Diseases (NTDs) programmes in planning and conducting assessment of black fly breeding sites and capturing adult black flies for onchocerciasis transmission assessments.

As a slimmed down, plain language, user friendly companion document designed for use by field teams, this toolkit does not include references to scientific publications. All scientific publications which this toolkit is built from are included in the Entomological manual for onchocerciasis elimination programmes.

This document does not replace the manual and should not be used without reference to the most recent version of the WHO document, by the trainers and programme managers prior to scheduling training (recommended training agenda is given in Annex 1).

WHO's Entomological manual for onchocerciasis elimination programmes is freely available to download by visiting this website:
https://www.who.int/publications/i/item/9789240068612

Or by clicking this link to directly download:
https://iris.who.int/bitstream/handle/10665/371688/9789240068612-eng.pdf?sequence=1

Chapter 2 - Overview of onchocerciasis

Onchocerciasis is a neglected tropical disease, NTD. It is a parasitic worm transmitted from person to person by the bite of infected female black flies. Black flies that transmit onchocerciasis are members of the *Simulium* species, mainly members of the *Simulium damnosum* complex, but members of the *Simulium neavei* complex in East Africa are also capable of transmitting onchocerciasis. An insect like a black fly that transmits a disease to people is called a vector. Black flies are vectors of onchocerciasis. Black flies reproduce in fast flowing, well oxygenated rivers and streams, typically where white water or rapids are found, but occasionally on dam spillways and other micro-habitats. Density of biting black flies, and therefore risk of onchocerciasis, is greatest closest to the sections of rivers in which they reproduce, giving onchocerciasis it's common name of river blindness. Onchocerciasis causes skin symptoms such as dermatitis, itching, loss of colour, premature aging/wrinkling of skin, and leopard skin, as well as visual impairment and blindness.

***Simulium* is the name of a genus of black flies. A genus is a group of related flies. You will see *Simulium* abbreviated to *S.* with the name of the black fly species following.**

Life cycle and transmission of onchocerciasis

The diagram below represents each stage of the lifecycle of *Onchocerca volvulus,* which is the full scientific name for the parasite that causes onchocerciasis/river blindness. The parasite lifecycle is divided between human stages and black fly stages as demonstrated in figure 2.1. Enhanced explanation of each lifecycle stage is included following figure 2.1, with numbers corresponding to those shown on the lifecycle.

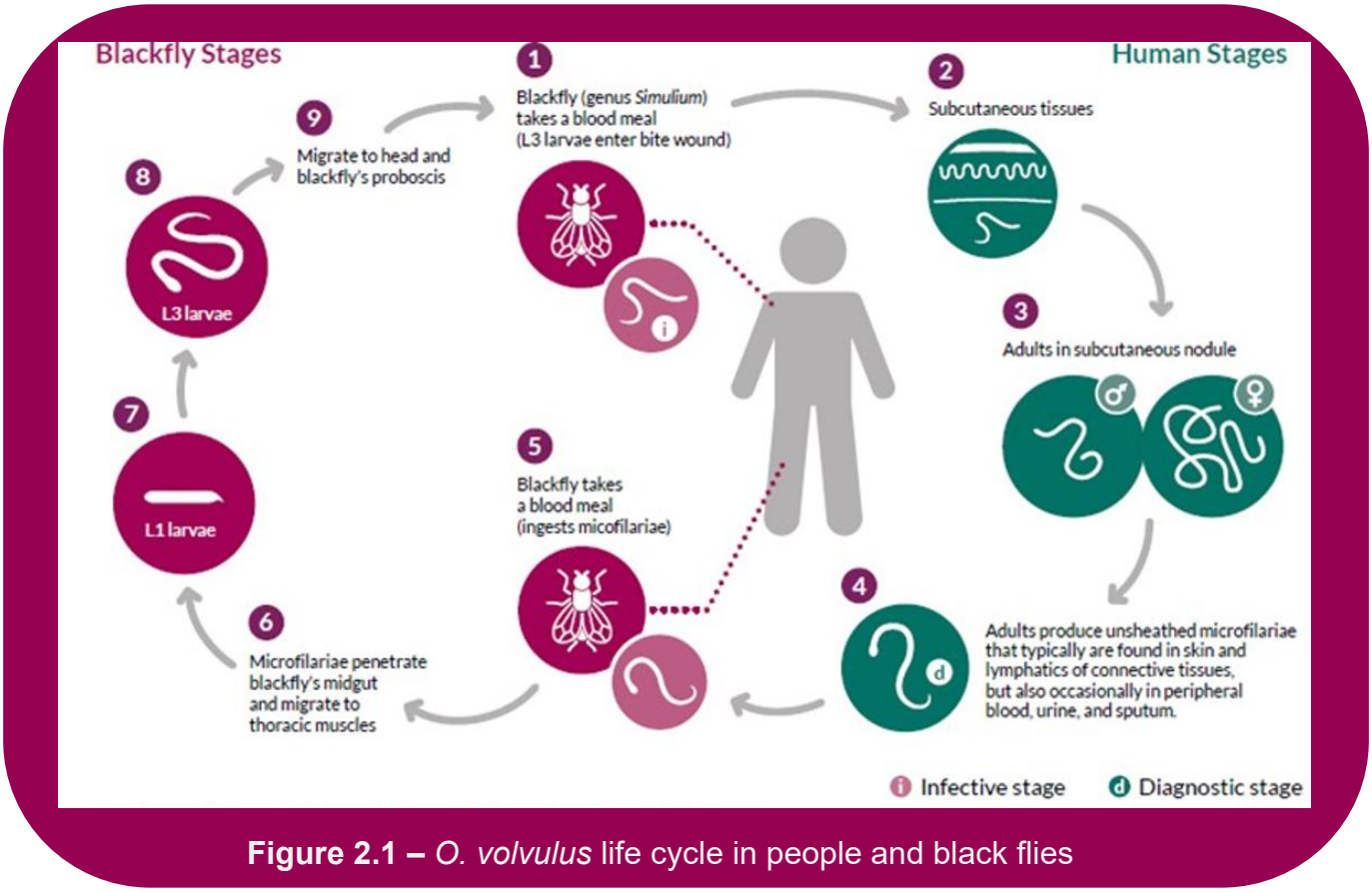

Figure 2.1 – *O. volvulus* life cycle in people and black flies

1. Onchocerciasis larvae L3 are transmitted to a person while the female black fly takes a bloodmeal.
2. On entering a person, the larval stages of onchocerciasis travel to the subcutaneous (just below the skin) tissues, and mature into adult worms, forming nodules. Nodules are basically worm nests beneath the skin. When they are near the surface or in a bony area, they are a visible, palpable bump under the skin. If they are in muscular or fatty areas, they can remain hidden, impossible to detect through physical examination. Mature female worms are 30 cm in length, males are much smaller at 4 cm long. It takes 12 – 16 months for maturation from L3 to adult worm to take place.
3. When the females mature, they begin to produce microfilariae (mf), which are baby worms. It takes around 18 months from becoming infected until the adult worms are mature and reproductively active. One single adult female worm can produce up to 1,000 mf per day. She can live for up to 15 years and be reproductively active for at least 12 of those years.
4. The mf produced by the adult female worm migrate from the nodule to the skin. The mf can live for up to two years in the skin.
5. A female black fly ingests mf in the skin of an infected individual when taking a bloodmeal.
6. The mf penetrate the black fly midgut and migrate to the thoracic muscles.
7. & 8. The mf change into larval stages of the parasite, and gradually mature from L1, the smallest stage, to L3, the stage which is infective to humans. The maturation process takes 7 – 10 days, maturation happens more quickly in warmer temperatures.

9. The mature L3 larvae migrate to the black flies head and proboscis (mouth parts). When the female takes a bloodmeal, the L3 will pass from the black fly into the human host, returning the cycle to step 1 again.

Major signs and symptoms

Onchocerciasis has a terrible impact on people who get symptoms. The mf are responsible for the majority of symptoms. Symptoms do not appear immediately, instead they are the result of prolonged infection, over several years. Given the long lifespan of adult female worms, and the number of mf they produce each day, extremely high numbers of mf can be present in the skin and other tissues, like the eye, exerting a pathological effect. The frequency and severity of symptoms are closely associated with the number of mf, which depends on the number of reproductive adults within an infected person and the duration of infection. Symptoms most commonly arise in the skin, resulting in a range of symptoms collectively known as “onchodermatitis”. Onchodermatitis includes, itching, rashes, inflammation and changes in the skin, premature aging of the skin and loss of elasticity (skin atrophy), depigmentation and leopard skin. Nodules in sensitive or bony areas, like over joints, or on the head, can cause pain and discomfort, but most nodules are not troublesome beyond the change in appearance caused by the bump under the skin.

When skin mf reach very high numbers, the risk of their invading the eye increases. Mf in the eye cause an intense immune reaction when they die, and this causes pathology such as lesions within the eye, visual impairment, and eventually, if untreated, blindness.

Social and economic impact

Globally, an estimated 1.15 million people (GBD, 2017) are blind because of onchocerciasis, almost exclusively residents in rural areas on very low incomes. As well as blindness the intense itching and other skin symptoms impairs the ability of infected people to normally participate in day-to-day activities. This reduces their capacity for employment, farming, or running a household, while also giving rise to significant health care costs. Onchocerciasis symptoms, and the difficulty in accessing and affording healthcare and paying for basic needs, have a profound impact on mental health and social well-being. As well as those actively infected, onchocerciasis has a large impact for whole households and communities, who have little support to provide care and assistance to those suffering from onchocerciasis symptoms. Consequently, healthy children miss school, and healthy adults must reduce their workload outside of the home to care for ill family members, or to seek additional employment to replace lost income from adults ill with onchocerciasis who are unable to work. Fertile riverside areas ideal for farming are underutilised in some areas due to the risk of onchocerciasis, amplifying the economic impact of the disease in endemic areas.

Treatment and elimination of onchocerciasis

Onchocerciasis has long been recognized as a public health problem. This resulted in the donation of Mectizan (ivermectin); medicine to treat the disease in endemic areas. Merck & Co. Inc. pledged to give the medicine away for free for the treatment of onchocerciasis, as much as needed, for as long as needed. Mectizan is a powerful microfilaricide. This means it kills the onchocerciasis mf but does not have a lasting effect on adult worms. Since mf are the transmissible stage, it is possible to break the transmission cycle by delivering repeated rounds of annual or biannual treatment with Mectizan to everyone living in at risk areas. By sustaining treatment for a long enough period, outlasting the adult worm lifespan of 12 – 15 years, elimination of transmission can be achieved. This is done through Mass Drug Administration, MDA. Since mf are also responsible for most symptoms, treatment with ivermectin quickly improves skin symptoms and reduces the risk of blindness at the individual level.

Notes on Chapter 2

Chapter 3 - Introduction to black flies

A major determinant of onchocerciasis risk is the location of a village in relation to the nearest black fly breeding site. This explains why black fly entomology forms such a central part of onchocerciasis elimination activities. Good knowledge of the black fly vector is essential to be able to measure whether onchocerciasis elimination has been achieved.

Black flies are members of the family Simuliidae, order Diptera. The family consists of over 2300 species, the genus *Simulium* being the largest (more than 1800 species). The insects are usually small, black or grey, with short legs and antennae. There are more than 55 different cytospecies in the *Simulium damnosum* complex, some more efficient at transmitting onchocerciasis than others. They are differently distributed across sub-Saharan Africa according to their habitat and host preferences. Only female black flies feed on blood, blood feeding is done to acquire protein, vital for producing eggs. Male black flies feed only on nectar from flowers, which is also how the females source their nutritional requirements. Adult female black flies are usually small (2–4 mm long), robust and dark, with a stout body and a characteristic humped thorax, as depicted in figure 3.1. The head has conspicuous compound eyes, which exhibit sexual dimorphism, apparent in figure 3.2.

Figure 3.1 – female black fly caught in Malawi

General lifecycle

Both male and female black flies regularly feed on plant juices and naturally occurring sugars, and only females feed on blood. A single blood meal provides sufficient nutrients to develop one batch of eggs. All species of the *Simulium damnosum* complex have the same basic lifecycle, set out in figure 3.2.

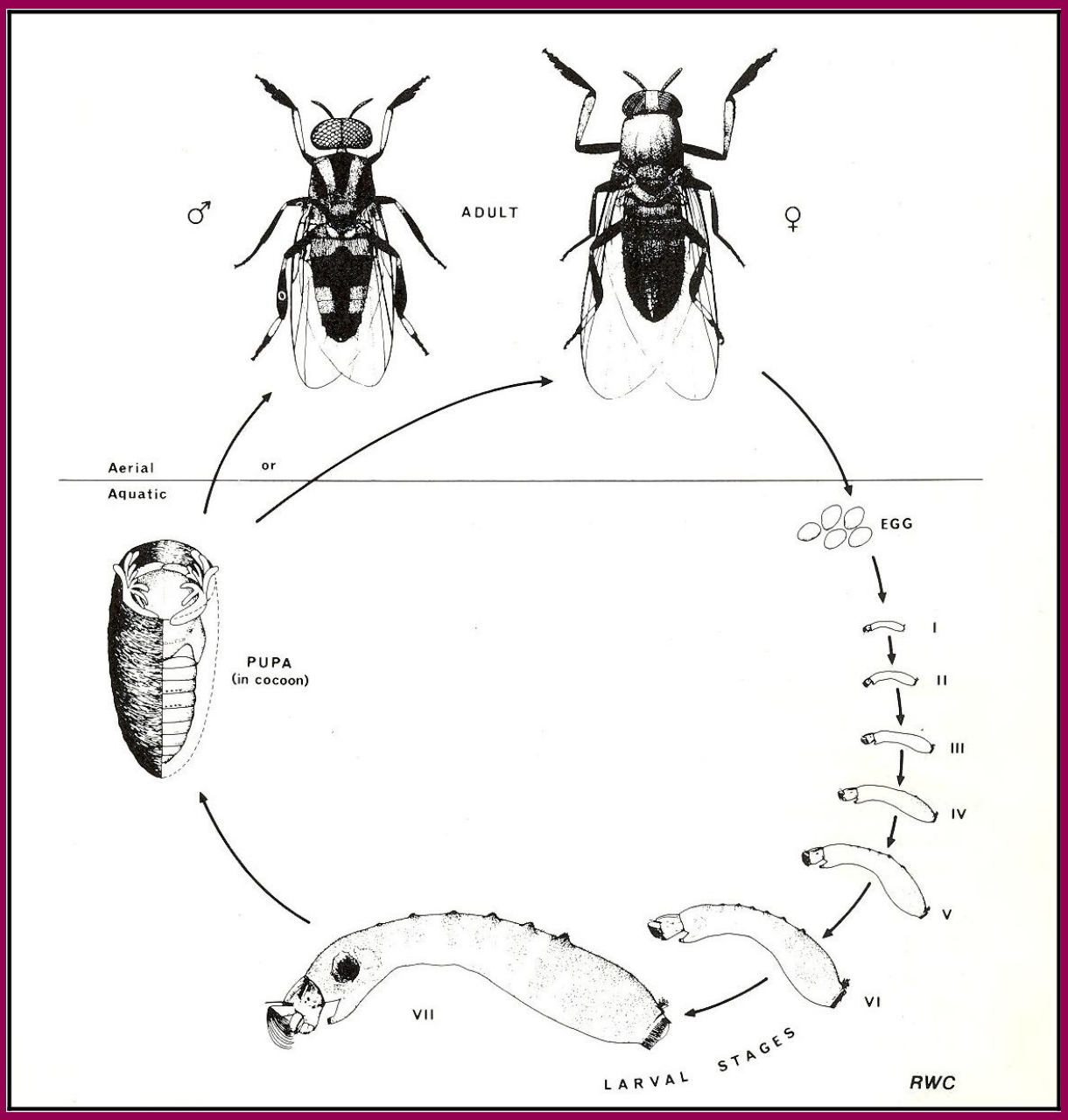

Figure 3.2: basic lifecycle of *Simulium* species (source: WHO 2023)

There are four distinct lifecycle stages for all *Simulium* species; egg, larvae, pupa and adult. All lifecycle stages except the adult are aquatic. Eggs are laid by adult females into running water, attached to vegetation within the water or very close to it. Eggs hatch into larvae after 1 – 3 days. To allow for growth, the larval stage sheds its skin (cuticle), including the hardened head capsule, several times. Between each skin shedding (moult) the larva is in a particular instar of it's development. After emerging from the egg, the larva is in it's 1st larval instar. Following the first moult, it becomes a second stage larval instar. Members of the *Simulium damnosum* complex have seven larval instars, with 1st instar being smallest and each subsequent instar getting progressively larger than the last. Passing through the seven larval instars usually takes 7 – 12 days. After the 7th instar, the larva moults a last time and transforms into the pupal stage. Within the pupa, the adult fly develops. After an average of 2

– 5 days for the *S. damnosum* complex, the adult black fly emerges from the pupae under water, floats to the surface and takes flight to mate, feed and continue the life cycle. Depending on local conditions, the entire process from egg to adult black fly takes 10 – 18 days to complete.

How to recognize *S. damnosum* complex eggs, larvae, pupae, adults

Eggs

Eggs have a typical ovoid shape, measuring between 0.1 – 0.4 mm in size. They are white when first laid, darkening to brown as time passes and development advances. The older the eggs, the darker the colour.

Larvae

The larva is easily recognizable by its characteristic shape. The body is long, slightly curved, and swollen at the rear end, where there are two attachment organs. They are often a dark colour and densely covered in setae all over the body. They have a proleg with hooks on the thorax, on the underside of the body and close to the head capsule. In *S. damnosum* s.l. the proleg will also have setae, unlike other African black fly species. The surface of the larva (the body cuticle) is either bare or with small, sparse, hairs or scales. The dorsal outline of the body is usually smooth, with dorsal protuberances called tubercles which stick out slightly on the first five or six abdomen segments. The head capsule is very sturdy, darker in colour than the body, with a pair of filamentous fans which are round when opened. On the upper surface of the head there is a pair of three-segmented antennae. Each side of the head there is a pair of small black pigmented marks called eye spots. Older larvae show histoblasts (dark, oval spots) of developing legs and wing on each side of the thorax, see figures 3.3 and 3.4.

Simulium damnosum typically has seven larval instars (figures 3.2 and 3.3) separated by moults. Each larva clings to a support with its posterior crown of hooks and a silken thread for anchors. Larvae may move within a stream by drifting in the current to new locations.

Figure 3.3 – magnified image showing early 6th, 7th and late 7th instar of black fly larvae. (source: WHO, 2023/R.J. Post)

Figure 3.4 – unmagnified black fly larvae (circled), pupae (square) and pupal cases (triangle) attached to vegetation removed from a river in Malawi (source: L. Hamill).

Pupae

At the end of the larval stage, the last larval instar spins silk secreted from the salivary glands into a protective, slipper-shaped, brownish cocoon and moults inside to a pupa, see figures 3.4 and 3.5. This stage remains anchored to the same substrate as the larva. Mature pupae generally look like adults wrapped in a membrane. They are yellowish when young but become increasingly dark as they develop. Pupae have a V shape with rounded top where the pupa protrudes from the cocoon. The abdomen of the pupae is not visible unless removed from the cocoon, and contains nine segments and several hooks to secure the pupa in the cocoon. Pupae do not feed and are completely embedded inside a cocoon, fixed at the support, with the exception of the respiratory filaments.

Figure 3.5: diagram showing blackly pupae and photograph showing pupae attached to vegetation (source: WHO, 2023/ left Dr Wolfgang Lechthaler, Eutaxa.com; right Carlos Pradera, Desinsectador 08-2017.)

Adults

Nearly all *Simulium* spp that feed on human blood belong to the genus *Simulium* s.l., and this is the only genus with human feeding members that is present in onchocerciasis endemic areas. Therefore, any human blood seeking black flies caught in endemic areas matching the description below belong to the *Simulium damnosum* complex.

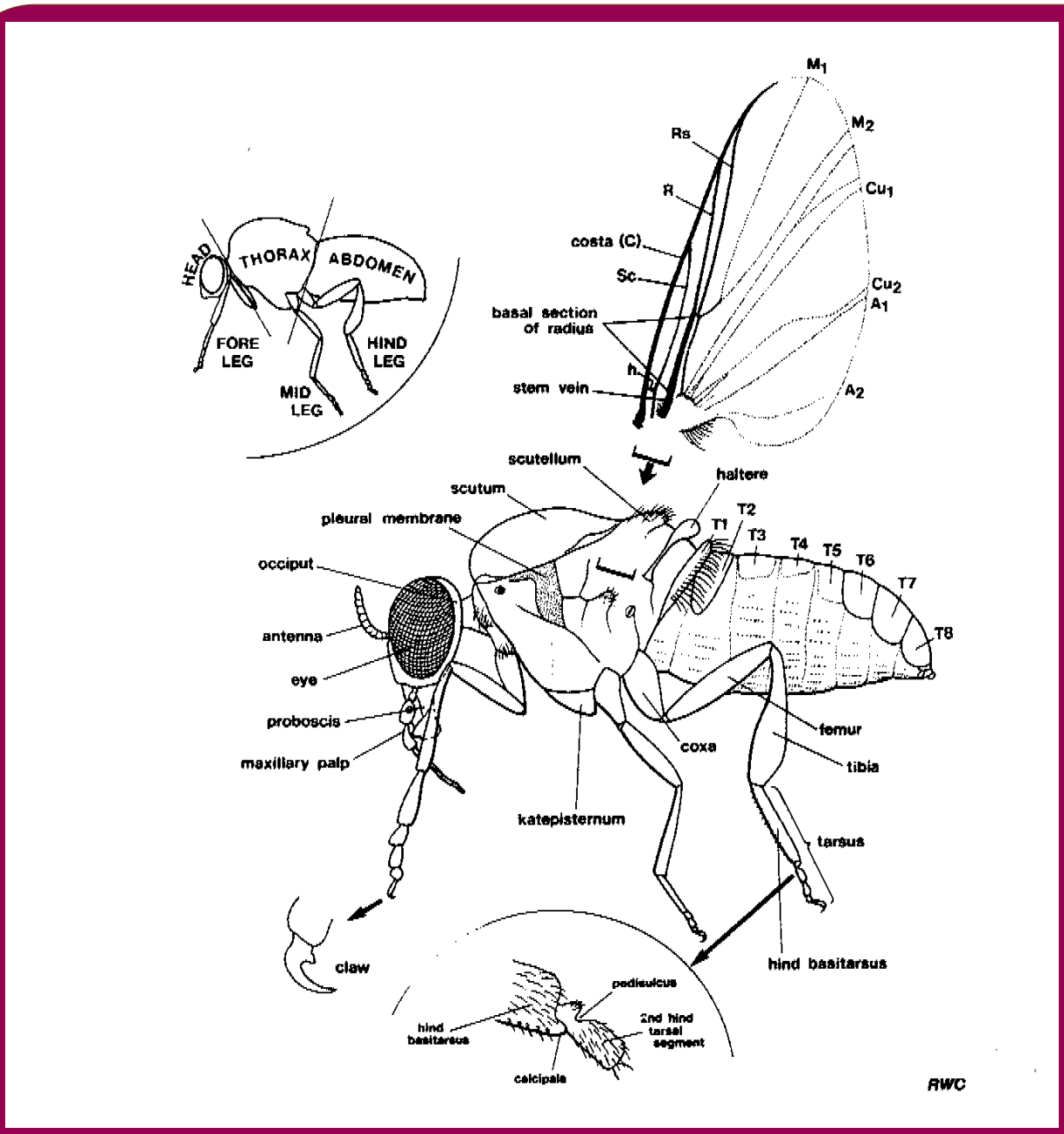

Figure 3.6 - key morphological features of *S. damnosum* black flies (source: Davies and Crosskey, 1991).

Black flies have a characteristic “hump backed” appearance from the side profile, as shown above in figure 3.6. The antennae are short and cigar-shaped, with 11 almost equal segments and without whorls of long hair seen in mosquitoes and other insect species. The mouthparts form a short downwardly directed proboscis. The wings are broad, colourless and without scales, they are broadest near the base. There are two characteristic large membranous areas on the thorax, located on each side on front of the wing base, they are called the pleural membranes. The abdomen is short with 9 distinct segments. On the first segment of the abdomen is a basal scale bearing a hair fringe. The legs are short and stout without scales.

The above features may be difficult to appreciate without a microscope or hand lens. When these are not available, the following more basic guide can help identify suspected black flies at the point of capture, which can then be confirmed using a microscope when transported to a lab. Female black flies should have:

- A dark brown or black body
- A humpback appearance when viewed from the side
- Filiform, relatively straight antennas which have a cigar like shape, 11 segments and are segmented. NB – sweat bees look very like blackflies, but they have geniculate antennae – with a bend or curve in them. Flies with curved antennae are not blackflies. Blackfly antennae are without hairs.
- Downward facing mouth parts
- Transparent wings without scales
- A short abdomen with 9 segments

Host seeking behaviour of female black flies

Female black flies use a series of visual and scent cues to first find and then move towards a host. Long-distance orientation begins after detection of colour and movement of the host. Darker colours (black, blue) are attractive to black flies. Once a potential host is seen, mid-range orientation is stimulated by carbon dioxide exhaled in breath, which brings the fly closer. The final set of host-seeking stimulants consists of a variety of chemicals present in sweat and on the skin of the host. Black flies feed on humans during the day and do not normally bite between sunset and sunrise. During the day, some cytospecies of black flies exhibit peak biting times – some peak around midday, while others have two peaks of biting activity, one just after sunrise, and one in the later part of the day as the sun begins to set. The biting cycle during the day may also vary seasonally and from place to place, so it is important that human landing capture and trapping activities are carried out for the whole day. Black flies can be sensitive to local weather conditions, biting rates will usually be lower during intensely heavy rain, windy conditions, and at high temperatures in full sunlight. Fly catching done for just one day will not be representative of local breeding, catches should be repeated for a few days per week for a few weeks to take into account these variations.

Breeding sites of black flies

The breeding sites for *S. damnosum* are always in fast flowing and well oxygenated water. These are often in white water rapids. The larvae and pupae can also live in niches away from white water sections such as cascades, waterfalls, lake outlets, dam spillways, small tributaries and springs. Black fly larvae can attach to almost any fixed object submerged in well oxygenated water. Black fly larvae are very sensitive to water pollution such as human or animal waste, or chemical runoff from agricultural activities; where larvae are found the water will be relatively clean. The locations within a river which have the optimal conditions

for reproduction and contain black fly larvae are known as breeding sites. However black fly breeding can occasionally take place outside of these typical “white water” breeding sites. For example, at the height of the rainy season, or when rivers are particularly full, a river may become so high and the flow of water so fast that rocky rapids are flooded and larvae breed in a dispersed manner on vegetation, particularly at the edges of the river, no longer concentrated at rapids. It can also happen in some savannah areas, when, at the height of the dry season, or after a prolonged period of no rain, a river may not run completely dry but maintain a trickle of flow in the main channel or channels. In these circumstances, larvae can be found in small clusters where the flow is fastest.

Exceptions aside, the typical black fly breeding sites are characterised by:

- The presence of fast-flowing water 0.4–2.4 m/s. On large rivers it might not always have to be where there is whitish or churning water but can be where the flow of the river is visibly quick.
- The sound of rushing water and/or water breaking due to a rocky bottom creating rapids,
- The availability of supports for the vector to lay its eggs on (e.g vegetation trailing in the water, roots, leaves, submerged rocks, emerged supports kept wet by water spray)
- The availability of nutrients in the water column (algae, plankton, diatoms, bacteria, plant detritus)
- Nearby community members complaining of biting flies.

Hydrological conditions for *S. damnosum* s. l. breeding sites are a temperature of 16–24^{0}C and a pH of 7.7–10.0 for the East African complex and a temperature of 22–33^{0}C and a pH of 5.7–6.2 for the West African complex. Low flow rate, food quality, predation and infection by microscopic pathogens regulate larval populations.

Examples of black fly breeding sites are demonstrated below, in figures 3.7, 3.8 and 3.9:

Figure 3.7 – a black fly breeding site in South West Cameroon (L. Hamill). Note the shallow depth of the water, exposed rocks and patches of white water, along with dense overhanging and trailing vegetation along the edge of the riverbanks. (source: L Hamill)

Figure 3.8 – a black fly breeding site on the Malawi – Mozambique border). Note the shallow depth of the water, exposed rocks and patches of white water, along with trailing vegetation along the edge of the riverbanks and on islands in the river. (source: L Hamill)

Figure 3.9 - a black fly breeding site on the Malawi – Mozambique border). Note the shallow depth of the water, exposed rocks and patches of white water, along with trailing vegetation along the edge of the riverbanks and on islands in the river. (source: L Hamill)

Summary

Black flies breed in swift-flowing, well-aerated or well-oxygenated rivers and streams, typically white-water areas. Only females of the species feed on blood, males feed on nectar from flowers. All lifecycle stages except the adult are aquatic. *S. damnosum* typically has seven larval instars, with the 1st instar being smallest and subsequent instars increasing in size. Black fly eggs are small, 0.1 – 0.4 mm in size. Larvae are long, slightly curved, with a pro-leg beneath the head capsule and swollen at the rear end, where there are two attachment organs. Pupae are "V" shaped and yellowish when young but become increasingly dark as they develop. Adult female black flies are usually small (2–4 mm long), robust and dark, with a stout body and a characteristic humped thorax. Depending on local conditions, the entire maturation process from egg to adult black fly takes 10 – 18 days to complete.

Notes on chapter 3

Chapter 4 - Community mobilisation

What is community mobilisation?

Community mobilization is essential for creating partnerships with various sectors of a community in order to address a pressing issue such as an NTD. Mobilization empowers community members and groups to act to facilitate change. Actions include identifying resources, providing information, generating support and fostering cooperation between public and private sectors in the community. Mobilization is often described as “building community or collaborative partnerships”, “community engagement” or “coalition building”.

Community participation during entomology evaluation

Community engagement and mobilization is very important for the success of public health programmes. Those carrying out entomological surveys or studies must mobilize endemic communities for successful entomological evaluation in onchocerciasis elimination. In such activities, endemic communities have significant roles to play in at least six areas.

(i) Identification of breeding sites: While the first step in identifying breeding sites is to consult maps, there may have been changes in the names of villages or rivers, diversion or branching of a river or relocation of villages. Sometimes, the characteristics of a river may have changed to such an extent that the local ecology no longer favours breeding of black flies, or the changes may have resulted in more or new breeding sites (e.g. construction of a dam). Local residents can be extremely helpful in guiding vector personnel to productive breeding sites, as they will be aware of the presence, abundance and seasonality of biting black flies.

(ii) The safety of breeding sites: Some river basins are not safe for entomological evaluation, for example, if dangerous wild animals such as crocodiles or hippopotamuses inhabit the river basin or a selected site hosts the shrine of village gods that forbid visits of foreigners or other groups. Some areas may be insecure because of village conflict. Villagers can inform the entomological team of such situations.

(iii) Prospection of breeding sites: Local residents can inform the team of the depth of the river for prospection, may assist the team in locating the aquatic stages of black flies or may confirm human biting activity of the female black flies.

(iv) Clearing and establishing capture points: Villagers may help the team in clearing and establishing catching points.

(v) Identification of locations for setting and protecting black fly traps: Villagers may be involved in identifying locations for collecting adult female black flies in traps. In some

experiences, children pulled traps apart and played with them, or traps were removed from sites. Villagers should be mobilized to “own” and protect traps.

(vi) Human landing collection of black flies at designated sites: For human landing collection (HLC) of female black flies, villagers may identify trusted members of the community to be trained as village vector collectors (VVCs), who have completed the information sheet (annex 4). Mobilization and involvement of leaders is necessary to identify members of the community who meet the criteria set by the entomology team to perform the role of VVCs.

Communities should be mobilized before the evaluation exercise. In all locations for evaluation, community leaders should be contacted and informed about the survey, including the objectives, the importance to health and the importance of community participation. Feedback from the community should be considered and the survey or prospection adjusted accordingly. Translation of key technical terms such as black fly and onchocerciasis into the local language should be done in advance of the mobilisation meetings.

Community Drug Distributors (CDDs) are usually recognized and respected by the community and should be involved in community mobilization. This should be discussed with village leaders in planning mobilization.

In areas without ongoing MDA, the team should initiate fresh community mobilization with appropriate onchocerciasis education materials, with emphasis on the evaluations to be undertaken in the villages and their purpose.

How to conduct effective mobilisation – practical advice and key messages for different activities.

The entomology team spend relatively longer time in the community, giving them opportunity to raise awareness on the following:

- Raise awareness of the disease:
    - What is onchocerciasis – link the black flies with blindness, itching, and ongoing MDA
- Raise awareness on how to reduce contact with black flies by providing information on peak biting times and use of locally available repellents.
- Provide awareness on linkage between disease and breeding sites of flies
- Importance of taking regular dose of ivermectin
- Awareness on qualification for ivermectin and exclusion criteria.

Notes on chapter 4

Chapter 5 - Practical steps for breeding site prospection

In Simulium ecology, prospection is the search for aquatic black fly stages (eggs, larvae, pupae) in potential breeding sites. Once the team arrives at a potential site, it must physically wade through the river or stream and locate and examine the substrates (rocks, trailing vegetation, aquatic plants, etc.) found in fast-flowing water for the presence of larvae and pupae. Identification of vector species allows the team to confirm the relevance of the site for breeding. In the context of elimination of onchocerciasis, confirmation of a breeding site allows identification and location of first line and second-line communities in which epidemiological evaluations or black fly collections should be conducted as part of the national surveillance strategy and in order to meet the WHO criteria for stopping MDA or confirming interruption of transmission through post-treatment surveillance.

Equipment required

In preparation for prospecting aquatic stages, the following are required:

- Map of the river system with potential breeding sites/prospection areas clearly marked
- Cold box
- Ice packs
- Knife, pruning shears or large scissors
- Hand magnifying lens
- Zip-lock plastic bags
- Notebook
- Pencil
- Thermometer
- Pencil and paper for labelling samples
- universal (Bijou) tubes or similar glass tubes with screw top lids
- Glacial acetic acid
- Ethanol
- Paper towels
- Mobile phone for GPS coordinate recording and data entry
- Life jackets for those who will enter water
- Waist belts for those who will enter water
- Clothes appropriate for prospection, particularly footwear that does not slip on rocks
- Walking stick for wading (this can be sourced on site)
- First aid kit (in case of injury).

An important aspect of prospection is community involvement, by consulting local residents for assistance in identifying and accessing potential breeding sites along with information on the safety of the river or the area.

Note: Prospection of high-volume rapids is dangerous and should not be done without proper training. Entomologists should never work alone. Life jackets and waist belts are not optional equipment.

Preparation of Carnoy's solution

Carnoy's fixative is a mixture of three parts of ethanol to one part of acetic acid, without water (i.e. 3:1 absolute ethanol to glacial acetic acid). The method described below is for fixing whole intact larvae and is the commonest and easiest method.

- In the laboratory, carefully measure out 21mL of absolute alcohol into a 30ml tube or bottle, and label.
- Carefully measure 7mL of acetic acid into 8mL bijou bottles or similar, and label appropriately.
- Transport all the bottles of absolute alcohol and acetic acid to the breeding site chosen for collection of larvae. Transport in a cool box with ice, but to prevent the liquids freezing do not place the bottles directly on the ice. The ingredients are transported separately as it is important for the Carnoy's solution to be freshly prepared so it can preserve the larvae well.

Basic steps in *S. damnosum* river prospection

- On arrival, inspect the river from the bank for potentially suitable black fly breeding habitat, and take photos. Look for any possible access to water. If there is no access, use machete to cut access to the river, taking care not to cut through crops or farmland.
- Check the river and surrounding area for safety. Do not enter the river if water is too deep or fast flowing, there are many boats/ferries, or if crocodiles or hippos are present.
- On entering the water search for white waters, over hanging vegetation, and trailing plants in the water. Remove submerged leaves, grasses, sticks and stones- then examine them for any attached larvae or pupae **on both sides**.
- In some sites where there are rocks, inspect rock surfaces and any movable substrate in the water that is close to the surface or in a shallow area of the river.
- At each section of the river 30-45 minutes should be spent examining submerged leaves, grasses, sticks and stones for larvae or pupae.

- If larvae are found, do not remove them from the substrate they are attached to. Instead, remove the leaf, twig or stick from the river and bring it back to the riverbank, and using a hand lens try to identify the larvae.
- Continue carefully moving from the side of the river into the middle, searching for black fly larvae. In larger rivers this may require a boat to reach islands or rocky outcrops
- Collect some larvae or pupa and while still in the field use a hand lens to inspect a larvae and check for the presence of large dark dorsal setae & tubercle, morphological characteristics of *S. damnosum* s.l. larvae. Setae on the proleg is characteristic for *S. damnosum* s.l. (see chapter three for more details). A dissecting microscope can also be used if available. Make brief notes on the characteristics observed in the larvae obtained from the river.
- **Important: examine larvae for their morphological features *before* preservation in Carnoy's solution.** Preservation and transportation of the larvae can remove the setae and make other morphological features more difficult to see.
- Take photos of larvae and if possible the location where they were found. Preserve a selection of larvae, (25 -50 individuals from a couple of sites/substrates in the river, selecting larger larvae where possible) in Carnoy's solution for future analysis. To do this, take the following steps:
    - At the edge of the breeding site (on the riverbank), open the tube or bottle containing 21 mL absolute alcohol retrieved from the cool box and add to it 7 mL of acetic acid from the bijou bottle, to obtain a final volume of 28 ml of Carnoy's fixative. Note: Gloves should be worn during this procedure to avoid contact with the final mixture. **Only do this once larvae have been found and are readily at hand for preservation.**
    - Close the cap tightly and shake to mix
    - Remove the substrate with larvae still attached from the river and blot it dry with paper towel, cloth, or other absorbent material. This is to avoid diluting the Carnoy's with river water.
    - Remove the larvae from the substrate with forceps and transfer them into the bottle of Carnoy's fixative. Then, start again with another substrate until the bottle contains 50 larvae. If there are more than 50 larvae, use a second bottle.
    - Label the bottle by writing on a small piece of paper in pencil the name of the river, place name, date and GPS coordinates, and the name or initials of the person collecting. Put this paper label inside the bottle with the flies and carnoy's, writing side facing out.
    - Prepare a second bottle of Carnoy's fixative. Pour away the old Carnoy's from the larval sample, and replace it with fresh fixative.
    - Put the bottle back in the icebox. Do not let water enter the bottle of fixative.
    - Transport the collected larvae in Carnoy's solution to the laboratory or storage place in the cool box.
    - Once at the laboratory, larvae can be kept refrigerated at 2 – 8 degrees for up to 2 years before analysis.

- Once a site on a river has been thoroughly searched, enter the results into the river prospection data collection form (Annex 4). Do not forget to take coordinates of the site using portable GPS or a phone with GPS application.
- Once the river has been searched, go to the nearest 2 - 3 communities and conduct the community questionnaire and first line village forms (Annex 2). Do this at all sections of river searched regardless of whether or not larvae are found.

Simulium neavei group breeding site assessment

S. neavei are only found in East and Central Africa. Prospections for the *S. neavei* group differ from that of *S. damnosum* because the larval stages of *S. neavei* attach themselves to freshwater crabs. In order to assess infestation status, freshwater crabs have to be caught and examined for immature stages of the vector. In preparation for the exercise you need to have:

Equipment

- Maps marked with suitable and uncertain areas from desk review
- Portable GPS machine or phone with GPS function
- Funnel basket traps
- Fresh meat/chicken or jack fruit
- Ropes
- Machetes
- Bucket
- Crab data collection form on ESPEN collect or paper form (annex 3)

Walk to the river course and inspect the site which has a good flow. Then along the riverbank:

- Prepare your basket trap by tying a small piece of fresh meat/chicken/ on a rope of about half a meter
- Insert the meat through the narrow side of the funnel basket trap and allow it to enter halfway. Then, block the narrow end with grass leaving the funnel open enough for ease of water flow
- With your trap already baited, carefully enter the water and select a suitable site in a river with moderate flow and shade
- Immerse the trap in water and cover it with grass or leaf to camouflage it since crabs are shy of bright objects. Use additional rope to anchor the trap to an object at the bank to avoid being swept by water current. Leave it in water for one hour
- In a river course, traps should be set at distance of 10-15 meters apart depending on the number of traps the field team has. The more the traps the better the results
- After one hour check if freshwater crabs have been trapped inside. If no crabs caught move to the next site

- If freshwater crabs are caught, remove the grass used for blocking entry and empty crabs in a bucket of clean water and examine them one by one for presence of immature stages (larvae/pupae) of *S. neavei*
- Results should be recorded in designated crab forms indicating the sex, size of crab, number of larvae, pupae and pupal cases
- If no crabs are caught completely in a river course, you can leave one or two traps overnight to rule the absence of freshwater crabs in the river/stream
- Before leaving the site record the coordinates using portable hand GPS or using a phone
- Once the river has been searched, go to the nearest 2 - 3 communities and conduct the community questionnaire and first line village forms (Annex 2). Do this at all sections of river searched regardless of whether or not larvae are found

Completing the community questionnaire on vectors

As well as breeding site assessment it is useful to collect information from communities residing along the rivers. In getting information about black fly, the team should be guided by key probing questions as there are many flies that exist along rivers in Africa that are not black flies. When completing the entomological community questionnaire (annex 2), the team should ask:

- If there are problems of “small black flies”, probe for size since tsetse flies are also common along rivers; show picture of black fly or actual one if available. (If you get answer NO from 5-10 people, then discontinue interview in the area)
- Painful bites that at times leave some spot of blood on the body
- Local name of black fly in the area if available
- How serious black fly bites are in the area– probe for preventing people from normal gardening activities etc
- Time of biting of the fly, -probe for early morning, afternoon or evening
- Which period of the year are black flies abundant? - Probe for season or months.

NOTE: People in the community who may have adequate information are fishermen, hunters, farmers or old opinion leaders. In the absence of these categories, any member of community can be interviewed.

Notes on chapter 5

Chapter 6 - Practical steps for human landing capture

Human landing capture is the current WHO recommended method for capturing host seeking female black flies to monitor and confirm elimination of transmission. It is, however, an outdated technique that requires humans to effectively act as live “bait” for insects known to transmit disease, morally and ethically this is extremely questionable. It is also very time consuming, deploying HLC at the scale required to verify elimination of onchocerciasis in Africa may not be logistically possible. Recent advances in black fly trapping (see chapter 6) mean that in the future there may be a replacement for human landing capture, but until that time, most onchocerciasis endemic countries will still need to conduct human landing capture. This chapter sets out how to conduct human landing capture in the safest and most efficient way. It should be noted that there is no such thing as zero risk when it comes to human landing capture. Therefore, before conducting human landing capture, the organisers should have sufficient funds to provide onchocerciasis treatments before and after the catches, and to support medical bills for any vector borne disease in HLC operatives for the duration of the catching period.

Materials needed

- Collection tube or pooter/aspirator
- Petrie dish
- 80% alcohol
- Pencil
- Paper
- Cotton wool
- Transparent tape
- Bijou or universal bottles holding 10 - 30ml total volume with a screw cap. Plastic tubes/bottles are acceptable
- Pastelle or similar device to decant alcohol
- Machete – to clear the site (this can be borrowed locally)
- Chair or stool to sit on
- Cool box with ice packs
- Mobile phone with ESPEN collect or paper data entry form (annex 3)
- Blue, black or grey umbrella or parasol (to shade the collector if the site is exposed)

Selection criteria for a collection site

Entomological monitoring sites are selected considering the epidemiology of disease in the area, the entomological conditions, and accessibility. Therefore, sites for conducting human landing capture to monitor onchocerciasis elimination should be:

- located in an area where onchocerciasis transmission was previously confirmed
- chosen near a productive and high-density breeding site
- in the shade and sheltered from the wind
- accessible all year round
- not a place of human gathering, to avoid both the dispersal of flies and the loss of attention of the collector
- The fly collection site should be located between any nearby communities and the river

Prior to training commencing, senior NTD officials will have selected the sites that need to be monitored. This task is not undertaken by field teams. For field teams, it may be necessary to clear vegetation or debris from a site before setting up for HLC. Make sure the environment is safe for the collectors to work in, and they are not at risk from injury caused by tripping or falling on uneven surfaces or overgrown vegetation.

Ethical considerations

Ethical clearance from local authorities should be obtained before the start of the study. In preparation for sample collection, sensitization and mobilisation activities will include contacting regional and district-level representatives, village chiefs and community health workers to explain the purpose of the black fly collections.

Informed consent

Anyone carrying out human landing capture must have given their informed consent. See annexes 4 and 5 for a template participant information sheet and consent form from which copies can be made for signing when human landing capture is about to be done. People should give their consent freely, without pressure or persuasion, they should have the process of human landing capture and the risks and benefits fully explained to them, they should have the chance to ask questions about human landing capture and their role, and they should be told they are free to withdraw at any time without negative consequences. Before a person can be enrolled to participate in human landing catches the informed consent procedure must take place.

Steps of informed consent:

- Tell the participant the purpose of the informed consent procedure and why it is important that it is done.
- Describe the study and their proposed participation including the risks and the benefits. (compensation is NOT a benefit)

- Emphasize that the study is voluntary. Refusing to participate carries no penalty. They also have a right to withdraw from the study without penalty at any time.
- Confirm that any and all data which is collected will not be shared in a way that means they could be identified.
- Be very clear of what activities the person will need to actively participate in and what you will put in place to protect them as much as possible from any risk.
- Make it clear that they can ask questions and ask for clarifications at any point if there is anything which they want to know or haven't understood, including contact details for someone to ask after the collections have started.
- Explain the compensation for their time that will be provided.
- Document the informed consent using the consent form. Complete two copies, one for them and one for the person taking the consent.

Selection of black fly collectors

The success of entomological surveys depends on the productivity of the collection sites and on the attention that the collector pays to the collection. For this the collector must:

- be competent to give informed consent for their participation
- be willing to participate and have given their informed consent
- if female, not pregnant or breastfeeding
- be professional
- have good reading, writing, and time keeping skills
- follow the schedule and places of collection
- be attentive and skilful
- be of good character and known in the community
- where possible, preference is given to agile, fit and healthy individuals, who will be able to complete the tasks well

To maximize community involvement in the work, the entomologist should ensure the following.

- Communities are properly informed about the importance of the entomological evaluation and study protocol.
- Community and opinion leaders can nominate potential fly collectors.
- Preference is given to residents of the community who are readily available for the task, which usually ensures that the residents have a sense of ownership and readiness to accept the outcome of the evaluation.
- The honorarium to be given to each vector collector should not be disclosed to the community leader(s) until selection has been completed.
- Fly collectors should be paid in instalments, according to local custom, to ensure that they remain motivated while conducting the work.

- All collectors should receive Mectizan® at least 1 week before collecting, and again immediately after the collections have ended. If possible, a five-week course of doxycycline should be given to each collector at the end of the catching period.

Training and supervision of community vector collectors

It is important that community vector collectors are taught to perform human landing capture well and safely. The training should be no shorter than half a day. Here are some key points to cover when training community vector collectors to catch black flies.

- Where will the fly catching take place? Ideally conduct the training at the location where catching will happen. If training several teams at once, train at one site, then accompany all teams to their own collection point and show them how best to set up catching at that specific point.
- When will the catching take place? On what dates will fly collection start and end on?
- How often will fly catch take place? How many days per week should collectors be active, and between which hours of the day? This information should be clearly given to all collectors. Explain that collectors should work in pairs between the hours of 7am and 6pm on collection days
- How to catch black flies? Review sections 7.6 and 7.5.1 with the trainees. Make sure they know what a black fly looks like. Ensure they understand how to catch the flies as they land, and the importance of preventing the black flies from biting them. Demonstrate how to do human landing capture, and the conduct a 30 mins test catch observing all collectors waiting for black flies and capturing them as they land. Advise collectors to expose only one leg, and cover the remaining leg, arms, and torso with loose fitting clothing. A long-sleeved t-shirt or other garment should be worn. These can be provided as part of the training if needed. This is to reduce the risk of collectors receiving bites from black flies or other insects like mosquitos on areas of the body they will not be able to capture the insect from.
- Train the collectors on how to preserve, label and store the black flies they catch each day. Show them how to enter data onto the mobile phone or paper data collection form. Tell them what the arrangements are for transporting the black flies to the laboratory for storage and analysis.
- Train more vector collectors than needed, so in case of illness or absence, collections can continue

Once training is completed, collections should start immediately. Always have training as close as possible to the planned activity the collectors are being trained to do, ideally the day before collection starts. On the day when catching begins, visit as many teams as possible, ideally all teams on the first day. Let the collectors know some dates when you will come for a supervision visit in future. Also plan to conduct some unannounced supervision visits, to make sure the collectors are performing well. During supervision visits, observe the activity and make any corrections needed to technique or positioning at the catching site. Take a few photographs on each supervision visit, so you can make a report of your supervision and the activities you observed during the visit, annex 6 gives checklist for supervision.

Making a clear identification of black flies

When observed with a magnifying glass, a black fly should have:

- A dark brown or black body
- A humpback appearance when viewed from the side
- Filiform, relatively straight antennas which have a cigar like shape, 11 segments and are segmented. NB – sweat bees look very like blackflies, but they have geniculate antennae – with a bend or curve in them. Flies with curved antennae are not blackflies. Blackfly antennae are without hairs.
- Downward facing mouth parts
- Transparent wings without scales
- A short abdomen with 9 segments

During the training, show the community entomology assistants labelled pictures of black flies, and preserved specimens if available, images are included in chapter 3 of this toolkit.

Method of black fly collection

- Each human landing capture team has two people who conduct the collection alternately.

Figure 6.1 – a VCC performing HLC in Malawi using a simple pooter (L Hamill)

- The collector should wear dark coloured, grey, blue or black clothing. The collector should not wear brightly coloured clothes
- The collector sits in the shade on a stool or other support with the trousers rolled up to the knees
- The collector should wear long sleeves to avoid black fly and other insect bites
- The blackflies coming to rest on the collectors are immediately covered in the collection tube (or using an aspirator), demonstrated in figure 6.1
- This will make the fly to fly or crawl up in the tube and then the collector will place his finger to close the tube before placing the lid to secure the fly.
- If using a pooter, the black fly can be placed directly into a bijoux bottle of alcohol by blowing it back down the tube while it is positioned over the bijoux bottle. Do not place more than 200 black flies in one tube or bottle.

- It is important to collect females before they begin their feeding process on the collector, to avoid loss of parasites and infection of the collector. This means collecting them as soon as they land
- Fly collection begins in the morning at 7am and ends in the evening at 5pm
- Each collector works for one hour in turn until the end of the day
- Once a collector has finished one hour, their partner takes over the collection while they count, label and preserve the black flies caught in 80% ethanol. No more than 200 black flies should be in a preservation tube. If more than 200 flies are caught take a new tube
- The number of blackflies collected each hour is recorded on the data collection sheet and/or app
- If collection tubes have been used, then at the end of each collection day, blackflies are transferred into preservation bottles containing 80% alcohol. There are several methods:
    - the tubes containing the blackflies are placed on ice or cool packs for 30 minutes to immobilise them, and then transferred to alcohol
    - The tube containing black flies is turned so the cap is facing downwards. Put your hand around the tube near the cap to make it dark so the black flies fly up away from the cap. Remove the cap from the Bijou bottle of alcohol first, then quickly remove the lid of the fly tube and hold the two together at their openings, fly tube upside down. Encourage to flies to go into the alcohol by carefully moving the tube and tapping on the up turned end, process depicted in figure 6.2

Figure 6.2 – blackflies being transferred from a collection tube to a preserving bottle (L Hamill)

- Carefully count how many flies are in preserving bottle. Do not put more than 200 flies in one preservation bottle, take a new tube.

- Do not mix flies caught in one hour with flies caught in a different hour. Each hour of collection should have its own preservation bottle.
- The samples are clearly labelled by indicating: - the village - date/time/period of collection - number of flies in the tube – name of collector
- Labels are written in pencil on a piece of paper that is inserted into the tube, see example in figure 6.3

Figure 6.3 - a preservation bottle containing 13 3 black flies and label written in pencil

Fly collection begins in the morning at 7 a.m. and ends in the evening at 5 p.m. Each collector works one hour in turn until the end of the day. (Example collector A from 7 a.m. to 8 a.m.; collector B from 8 a.m. to 9 a.m.; collector A from 9 a.m. to 10 a.m.; collector B from 10 a.m. to 11 a.m., in turn every hour until the end of the day). During their hour off, the collector rests and gets refreshment, and counts the number of black flies they collected during their previous working hour, and records this on the ESPEN collect app, or on paper if the app is not available.

Notes on chapter 6

Chapter 7 - Traps for collecting black flies

There has long been interest in developing a trap to replace HLC as the primary method for collecting black flies. The most promising technique to date is the Esperanza Window Trap, EWT. This was first developed in South America and later adapt for use in Africa. The trap is a 1m x 1m square piece of blue fabric with a black stripe down the middle, as shown in figure 7.1. The surface of both sides of the trap is coated with glue to immobilise any insects landing on the fabric. To better mimic host signals, the trap is baited with a carbon dioxide source and worn socks or trousers. The EWT is easy to construct, and all its components can be locally sourced.

Figure 7.1 – a pair of standard blue black blue EWTs deployed in Mozambique (L Hamill)

Practical steps in deploying EWT

Equipment needed:

- ordinary bakers yeast (*Saccharomyces cerevisiae*); 30g per trap side
- brown sugar (containing molasses); 500g per trap side
- water
- polymer tarpaulin 1m x 1m (blue with black strip in the middle)

- trap frame (wooden or metal frame and pegs)
- glue to coat the trap
- brush to apply glue
- rubber gloves
- rope
- scissors
- copper wire
- duct tape or other strong all weather adhesive tape
- 5 L plastic container
- 2.5 L plastic container
- weighing scales
- tubing
- forceps
- bijou bottles
- white mineral spirits (or kerosene)
- 80% ethanol
- human scent lure consisting of used, unwashed socks or chemical scent lure.
- field recording book or form on ESPEN collect or paper; and
- lead pencil (not an ink pen)
- torch
- cable ties

Notes on trap manufacture/construction

The tarpaulin used for the trap surface should be thick, waterproof and tear resistant. It should have the colour on both sides, or else two pieces stitched together with their coloured sides out. It should not be shiny or reflective, as this could repel black flies.

The frame can be made from a range of materials, depending on what is locally available and practical. Some examples are plumbing piping (light weight and easy to cut/ join), tent poles, wood, or metal rods welded into a square. When making the trap frames, the durability and ease of transport should be considered. Frame materials should not be so light that they would easily fall or get knocked over, or so heavy that they are difficult to carry. As well as constructing a frame upon which the trap tarpaulin is hung, it is also possible to stich the tarpaulin onto the trap, or have “sleeves” on the edge of the tarpaulin into which the poles or sticks can be threaded.

Procedure

1. **Preparation of organically generated carbon dioxide**:

- Weigh 30 g of dried yeast.
- Weigh 500 g of sugar.
- Measure 2.5 L of clean water.

- Mix all the constituents in a 5 – 10 litre plastic container that has a narrow opening that can be securely connected to the tubing using duct tape to capture all CO_2 produced and funnel it onto the top of the trap

Note: The organic carbon dioxide should be prepared at least 2 hours before starting trapping, and the sugar–yeast mixture should be changed daily. The sugar yeast mixture should be completely sealed apart from the CO_2 outlet tube, to prevent ants or other insects accessing the liquid.

2. Field deployment of the trap:

The exact method to deploy the trap depends on how it has been constructed. The basic steps are outlined below, to be adapted as required.

- Dig a few centimetres into the ground to erect the two pegs/poles 1 m apart at the selected trapping site. This is the frame that the trap will be attached to.
- Hook the trap tarpaulin firmly to the pegs at both ends with copper wire, rope, or string ensuring that the trap is 15 cm above ground level.
- Coat the two surfaces of the trap with adhesive using the brush. Only a thin coating is required, and over-application should be avoided. After application, the trap should be allowed to stand for at least 24 h before it is used for collection.
- Place the tubing connected to the plastic bottle containing the yeast–sugar mixture to
- release carbon dioxide to the trap. The tubing should extend to the middle of the trap, and should be connected securely to the side or top of the trap.
- Baiting the trap with the worn socks is done by placing the socks in a perforated nylon bag and hang it beside the trap. The nylon should not touch the glue. It should be removed daily at the end of trapping and brought back the next day.
- It is best practice to set traps in pairs, one parallel to the breeding site/river, and one perpendicular, in partially shaded or slightly exposed areas. Placement of the traps in totally shaded areas or excessively exposed sites may result in poor catches.
- TAD all weather glue is effective for up to 1 month, but regular monitoring of the condition and efficacy of the glue should be done, and glue that appears dry or has lost its “stickiness” can be spot refreshed or replaced.
- Old adhesive can be replaced by removing the adhesive with kerosene and applying fresh glue. Alternatively, an entirely new tarpaulin sheet can be used while the first one is taken away to be cleaned ready for recoating.

TIP: IF THE GROUND AROUND THE TRAP IS LOOSE OR SANDY, EMBED THE SUGAR YEAST MIX A FEW CENTIMETRES IN THE GROUND TO PREVENT IT FALLING OVER OR SPILLING

- Traps should be kept clean each day by removing any leaves, dirt or twigs trapped with forceps and kerosine.
- When not in use, traps can be protected by wrapping them loosely in plastic sheets so they do not collect flies overnight or on days when trapping is not scheduled to take place. Traps should also be protected when heavy rainfall is expected. When strong winds are expected traps should be taken down and moved inside, to prevent damage to the frame.
- On each day when the traps are scheduled to be active, the trap attendant should go to the trap site at 6am to make fresh sugar yeast mix for CO_2, replace the worn socks bait and remove the trap cover by 7am. The time the cover has been removed, and the trap is operational is recorded. At 6pm, the attendant should return to the trap to remove the day's catch and cover the trap overnight. It is important to do this each day so the daily number of flies collected can be recorded.

3. **Recovery of flies from the trap.** The steps in recovery of flies from the traps are:
   - Recover trapped *S. damnosum* once (at 17:00 h) daily using forceps. Collectors should wear gloves to prevent excessive contact between glue, solvents and skin
   - Pick all flies and other insects from the trap off using flexible forceps and put them in a plastic container containing kerosene (for TAD all weather) or white spirits. The type of solvent will depend on the glue being used.
   - Allow the sample to remain in the solvent for a few minutes, then gently wash off the glue from each insect trapped inside the container containing kerosene by holding them in the forceps one at a time and gently agitating them inside the kerosene.
   - Separate black flies from other flies and insects. If you are unsure if something is a black fly then count it with the black flies as entomologists will be checking the identifications.
   - Count the number of the black flies trapped, and transfer them to a bijou bottle containing 80% alcohol.
   - Count any other flies like mosquitoes, tsetse, etc, and preserve them in a bijou bottle containing 80% alcohol. No more than 200 flies should be included in a bottle, depending on the size on insect it might need to be significantly fewer than 200. There is no need to separate the other insects, black flies should be separated and everything else can be collected together for separation in the lab or field processing site.
   - The samples are clearly labelled by indicating: - the trap type - trap position (parallel or perpendicular) – trap side (perpendicular: side a is facing upstream, side b is downstream, parallel side a is facing the river, side b is facing away) - the location - date/time of collection - number of flies in the tube – name of collector
   - Labels are written in pencil on a piece of paper that is inserted into the tube

Note: One person can operate two or three traps effectively each day.

Notes on chapter 7

Annexes

Annex 1: Training Agenda

Time	Activity	Person Responsible
	Day 1, Thursday	
9.00-9:15	Introductions and orientation	Malawi Country Director
9.15-9:30	Welcome	Oncho Programme Manager, Malawi, MoH
9:30-10:00	DISSECT overview recap	Martins Imhansoloeva
10:00-10:15	**Coffee Break**	All
10:15-12:15	Finalising precise definition of the project area	Louise Hamill
12:15-13:15	**Lunch Break**	All
13:15-14:00	Presentation: draft breeding site assessment toolkit	Louise Hamill
14:00–16:00	Feedback and discussion of draft breeding site assessment toolkit in small groups	Adebiyi Adeniran Martins Imhansoloeva
16:00–16:45	Report back to main group, agreement on changes needed and next steps	Adebiyi Adeniran
16:45–16:55	**Coffee Break**	All
17:00	**End of day 1**	
	Day 2, Friday	
9.00-9:30	Welcome and recap from day 1	Nelvina Mucato
9:30-10:00	Presentation of the draft transmission assessment toolkit	Adebiyi Adeniran
10:00-10:15	**Coffee Break**	**All**
10:15-12:15	Feedback and discussion of draft transmission assessment toolkit	Martins Imhansoloeva Mercia Cumaio
12:15-12:30	Report back to main group, agreement on changes needed and next steps	Mercia Cumaio John Chipeta
12:30-13:45	**Lunch**	**All**
13:45-14:45	Presentation and feedback on Community mobilisation	Martins Imhansoloeva Adebiyi Adeniran
14:45–15:45	Review and feedback on implementation timeline	Louise Hamill
15:15–15:30	**Coffee Break**	**All**
15:45–17:00	Next steps	Martins Imhansoloeva
17:00	**End of workshop**	
	Day 3, Saturday	
08:30am	Visit to collaborating lab Zomba Central Hospital Laboratory	Louise Hamill Martins Imhansoloeva Adebiyi Adeniran, Sight savers MCO

Annex 2: Entomology community questionnaire outline for ESPEN collect mobile data collection

No.	Question	Explanation
1	Recorder ID	Enter 2-digit recorder code provided to each recorder (person doing the data entry)
2	Select district	Select the appropriate district
3	Site	The name of the community you are in, text entry
4	GPS	Collect GPS coordinates
5	Does this person consent to take part in the study?	Yes, No. select 1, No will end the survey, give warning before exit
6	What gender is this person?	Male, Female, select one
7	What age is this person?	Number entry
8	What is their occupation?	Text entry
9	How long has this person lived in this community?	Number entry
10	Black fly problem	Ask if black fly bites are a problem in the community Yes | No | Don't know
11	Bites number	If yes, how many bites do you receive each day? How larvae where found? 1 – 10 11 – 50 More than 50 (select one)
12	Black fly month	Select the month or months when the respondent reports black fly biting is most frequent
13	Black fly time of day	Morning, midday, afternoon, evening
14	Notes	Optional additional notes

Annex 3: River breeding site assessment form for ESPEN collect mobile data collection

No.	Question	Explanation
1	Recorder ID	Enter 2-digit recorder code provided to each recorder (person doing the data entry)
2	Select Province	Select the province you are working in from a drop-down list
3	Select district	Select the appropriate district from drop down list
4	Site	The name of the nearest community, text entry
5	Enter today's date	DD/MM/YYYY
6	GPS	Collect site coordinates: must be done outside
7	River	The name of the river, stream or tributary
8	River Basin	The name of the river basin
9	Inspection time	At time of inspection, did site appear suitable for black fly breeding. Yes | No.
10	Larvae evidence	At time of inspection, did site provide evidence of larvae. Yes | No
11	Abundance	How larvae where found? 1 – 10 11 – 50 More than 50 (select one)
12	Notes	Optional additional notes

Annex 4: Participant information sheet for village vector collectors/human landing catchers (VCCs)

Purpose of this activity:

My name is [insert name]. We are here today from the Ministry of Health, working in partnership with Sightsavers. We have come to check for river blindness in this community. River blindness is an infectious disease spread by the bite of black flies. It can cause your skin to itch and it can make you go blind. We want to know if there are any black flies in or close to where you live, how many there are and how frequently they bite people. These flies reproduce in fast flowing rivers. Today we are here to conduct training for village vector collectors to become proficient in the methods for catching adult black flies. After that village vector collectors will conduct black fly catch at regular intervals agreed between them and the training team

Methods:

If you agree to help in the collection, your help will be needed 1 or 2 days a week for up to 3 months. You will be asked to provide some basic personal information such as name, age and occupation. The information you provide will be stored confidentially. You will then be shown some written and practical training materials instructing you how to safely capture adult black flies. If you successfully complete the training, we will ask you to take a medicine called Mectizan, and then you will be asked to conduct black fly collections on a set number of days over the next three months. Black fly catches will be done for 5 hours in the morning and for 5 hours in the afternoon. During each catch period, you will sit on a stool or chair and capture any flies landing on you with a suction tube. This involves exposing one of your legs while sitting close to the river to attract black flies, and then capturing any black flies that land on your leg before they have the chance to bite you. You will work as part of a team with one other person, taking it in turns to spend one hour catching flies and one hour sorting and counting the flies caught in the previous hour. Lunch will not be provided as part of this collection. At the end of the day, all flies collected should be labelled and stored until collection at [insert day and time] by [insert name of person]. At the end of the catching period, we will ask you to take a medicine called doxycycline for a total of five weeks after the last day you completed catching on. Remember that it is important that you continue to take the pills against river blindness when they are distributed in your village.

Risks:

The risks of participating in this study have been minimized as much as possible. You may experience some discomfort if you are accidentally bitten by a black fly or other insect. The training you will receive will teach you how to avoid this. There is a small risk that if bitten, you could pass on onchocerciasis parasites to a black fly that will go on to bite another person. To avoid this, we will ask you to take Mectizan before starting the catching. There is a small risk, if bitten, you could contract a new onchocerciasis infection from an infective black fly. To avoid this, you will be given a course of doxycycline immediately when catching ends. Doxycycline is a curative treatment for onchocerciasis if taken for the full five weeks. There is a small risk you could contract a new malaria infection, if bitten by a mosquito while

sitting by the river. If you get malaria we will provide the pills to treat it at no cost to you. If you become sick during your work for the study we will help you get to a doctor. If your illness is caused by bites you got during your work, we will pay for your treatment. If you experience a new illness any time, even if it is not on a catching day, please go to the nearest health centre at [insert health centre name] for treatment and diagnosis, and contact [name of local coordinator, phone number, e-mail address] to notify them of your illness.

Right to withdraw:

Your participation in this study is voluntary. You do not have to take part in this survey. If you do join, and later change your mind, you may quit at any time. If you want to quit, please let us know by contacting [coordinator name, phone number, e-mail address]. If you refuse to join or quit early, you will not be punished or lose any benefits to which you have a right. If you decide to quit, you will not lose access to any benefits; you will be paid for each day that you worked. You will still be able to get medical treatment if you get sick because of the work you did for the study.

Benefits:

There will be no direct immediate benefit for participating in this study. However, by participating in this study, you will contribute to our understanding of river blindness and help us plan future activities and treatments to successfully control and eliminate river blindness here.

Compensation:

We will compensate you for your lost time spent at the river on catching days. This will be paid at a rate of [insert daily fee] for each days catching completed.

Data protection:

The Ministry of Health and Sightsavers will take a number of steps to keep any personal information about you private. Reports created will not identify you by name. The entire survey team is required to keep your identity confidential. The information that identifies you will not be given out to people who are not working on the survey.

Who to contact:

The information on this disclosure statement explains the rights to which you are entitled by joining this survey. If at any time you have questions about the research study, or if you experience any problems with a member of the study team or the study methods, and wish to make a complaint, you may [NTD or onchocerciasis coordinator name, official job title], at the Ministry of Health; [contact phone number and e-mail address]. If you have questions about your rights as a study participant, you may contact the [name of ethics review board chair that approved the survey]:

Name:

Building:

Street:

City:

Phone/e-mail:

Annex 5: Consent form

Survey title: Entomology transmission assessment in [IU name], [country name]

Consent to give personal information, complete training and carry out duties as a village vector collector

CONSENT:

This person has been informed of the nature, purpose and risk of the procedures described in the participant information sheet. He or she has been given time to ask any questions and these questions have been answered to the best of the investigator's ability. A signed copy of this consent form will be made available to the participant.

_____ _____

Investigator's Signature Date

I have been informed about this research study, its' possible benefits, risks, and discomforts. I hereby agree to participate in this study by completing training, receiving protective medication, and completing regular black fly collections between the hours of 7am and 6pm on designated collection days within my village. I recognize I am free to withdraw this consent and quit this project at any time, and that doing so will not cause me any penalty or loss of benefits that I would be otherwise entitled to enjoy. I have had the chance to ask questions and all of my questions have been answered. If I have any new questions, believe that I have been hurt by working with the study team, or want to stop working with the study team, I can call [insert name, phone number] from [insert name of institution].

_____ _____

Participant's Signature Date

Annex 6: Checklist for supervision of human landing collection

Name of community: **Name of supervisor:**

District/Province: **Name of collectors visited:**

Country: **Date of visit:**

This form must be completed by each supervisor during HLC supervision visit

S/No.	Checklist for Supervision	Yes	No	Comment (If No)
1	Did you meet the VVC* at designated catching point?			
2	Did you observe the VVC conducting fly capture in the field in accordance with the protocol/SOPs?			
3	Were the flies well preserved in 80% alcohol?			
4	Did VVC account for the flies caught at previous days?			
5	Were all preservation bottles well labelled with community code/name and date(s)?			
6	Were all entomological forms well and fully completed?			
7	Was there shortage of catching or preservation materials? If yes, what were the steps taken (comment)?			
8	Were there challenges or security threats? If yes, what were the steps taken (comment)?			

* Village Vector Collectors

Name of Supervisor ……..

Signature and date ……