

Vector studies for water-related diseases

Authors

Eline Boelee, International Water Management Institute, Ethiopia

Hammou Laamrani, International Development Research Centre, Egypt / International Water Management Institute, Ghana

Mekonnen Yohannes, Mekelle University, Ethiopia

Jean-Noel Poda, Institut de Recherches sur les Sciences de Santé (IRSS), Burkina Faso

Jean-Bosco Ouedraogo (IRSS), Burkina Faso

Dramane Zongo, Université de Ouagadougou, Burkina Faso

Based on work by: Felix Amerasinghe† (IWMI), Philippe Cecchi (IRD), Roch Dabire (IRSS), Gayathri Jayasinghe (IWMI), Henry Madsen (DBL) and references.

Scope: questions/ challenges the tool addresses

One of the health risks of small reservoirs is the potential for increased transmission of water-related diseases, in particular, parasitic infections dependent on water-based “vectors”. These are defined here as organisms that find suitable breeding sites in or around small reservoirs and either directly transmit parasites (for example, *Anopheles* mosquitoes for malaria) or serve as intermediate hosts (for example, *Bulinus* and *Biomphalaria* snails for schistosomiasis). The design, use and management of water bodies all influence their suitability as breeding grounds for disease vectors.

While actual transmission of water-related diseases depends on many factors, the presence of vectors determines the *risk* of transmission. Understanding the ecological preferences of vector organisms in relation to small reservoirs is an important step in identifying management options for environmental disease control. By controlling vector populations (referred to as “source reduction”), transmission risk is reduced. This can complement more conventional treatment-based disease control strategies.

One way to evaluate the risk of disease transmission through vectors is to conduct cross-sectional surveys of a sample of water bodies in and around selected reservoirs, communities or schools. Water bodies can be categorized (for example, reservoirs, canals, drains, seepage areas, pits, rain puddles), mapped, and vector incidence measured in a baseline inventory. A sample of these water bodies can be monitored (monthly or quarterly) for mosquito larvae and snails. Seasonal differences can be measured, and vector incidence can be related to water use and management practices. Control sites can be established in comparable communities without small reservoirs.

Sampling for *Anopheles* mosquito larvae is done with standard dippers (350 mm), with the number of dips depending on the size of the site (Amerasinghe et al. 2001). Aquatic snails in deep water bodies are sampled quantitatively using a drag scoop. In shallow habitats, quadrates are sampled, with sample size depending on surface and morphological variation in the sites

(Laamrani et al. in preparation). Adult mosquitoes can be captured in various ways, e.g. by indoor and outdoor spray catches, by netting sweeps of the vegetation, by human or animal bait catches and by light traps. The latter methodology is standardized and the most widely accepted.

If resources allow, mosquitoes and snails can be checked for infection with the relevant parasite. Methods are described below for checking snails for schistosomiasis, *Anopheles* mosquitoes for malaria. In addition, tests are available to determine the type of blood meal (human, animal) the mosquitoes have ingested. More information on other water-related diseases, their vectors and parasites can be found in the literature.

The fact sheets of the World Health Organization on water-related diseases are a good starting point:

http://www.who.int/water_sanitation_health/diseases/diseasefact/en/index.html

The American Centers for Disease Control and Prevention have good overviews too:

On mosquito-borne diseases at:

http://www.cdc.gov/ncidod/diseases/list_mosquitoborne.htm

On schistosomiasis at:

<http://www.cdc.gov/ncidod/dpd/parasites/schistosomiasis/default.htm>

And on other insect- and arthropod-related diseases at:

<http://www.cdc.gov/ncidod/diseases/insects/index.htm>

Target group of the tool

Target groups for this tool include planners, designers, builders, managers, and users of small, multi-purpose reservoirs who wish to increase health benefits and reduce health risks from these reservoirs. It may also be of use to health professionals and researchers.

Requirements for tool application

This tool requires participation by health care professionals, typically from government research institutes or universities, and entomologists familiar with medical entomology.

Material needed include: light traps (with batteries or chargers), sampling scoops, nets, hand-held GPS, pH & EC meters, boots, gloves, sampling pots, microscope.

Tool: description and application

Breeding sites

At the beginning of the study, different types of water bodies (for example, reservoirs, canals, drains, seepage areas, pits, puddles) at dam sites and in nearby villages are identified and mapped. In a baseline inventory, measurements are taken for all water bodies. After that, a sub-set of water bodies is monitored on a monthly or quarterly basis. In each round of monitoring, breeding sites are described with respect to substratum, (aquatic) vegetation cover, presence of fauna, turbidity, apparent pollution, and exposure to sunlight based on visual observation. Water temperature, pH and electrical conductivity are measured.

In addition, actual water use or signs of previous water use are described, to help determine principal water use points. The coordinates of potential vector breeding sites are recorded using a handheld GPS. Samples are collected between 9:00 and 16:00 hrs. At the end of the wet season, a new inventory of water bodies is conducted to ascertain whether new breeding sites have developed. Examples of data sheets that have been used in Sri Lanka for monitoring reservoirs (known locally as “tanks”) are shown at the end of the document (Amerasinghe et al. (2001).

Mosquito larval sampling

For monitoring of mosquito larvae, sampling should be carried out twice a month if possible, but at least quarterly. According to the size of the water body a representative number of dips are taken from each type of breeding site using standard dippers (350 mm). In small sites (< 10 m²) 6 dips per square meter are taken (Amerasinghe et al. 2001). A maximum of 10 dips is taken from each site. If breeding sites are too small for dipping more than once, only one dip per pool or site is made and additional dips are taken from nearby similar pools until a maximum of 10 dips is obtained from each type of breeding site. In larger sites such as reservoir margins, quadrates of 0.5m x 10 m are sampled. For each breeding site, the number of dips and the number of mosquito larvae per dip are recorded on special field collection sheets (see an example at the end of this document). Each breeding site will be recorded as “negative” or “positive” during each round of sampling.

All larvae are preserved in alcohol and larval stages III and IV classified by genus. *Anopheles* aquatic forms are identified by species according to Verrone (1962b) for Ethiopia or relevant keys for other countries. Some of the pupae can be reared to the adult stage for confirmation of species identity.

The categories and characteristics (physical and chemical) of breeding sites are noted and their location recorded during each round of sampling. Physical and chemical characteristics such as the presence or absence of emergent and floating vegetation, turbidity, pollution levels, temperature, electrical conductivity and pH are also recorded.



Figure 1. Mosquito larval sampling in Tigray, Ethiopia; left at Mai Sesella (Dr. Mekonnen Yohannes left with the white shirt) and right at Mai Negus (photos by Eline Boelee).

Larva Form as prepared for Tigray, Ethiopia (4 tables fit on a landscape page)

DATE		REF		LOCALI	
HABIT		AREA		DIPS	
TIME		LIGHT		BOTTO	
TEMP		WATER		MOSQ	
VEG					
FAUNA					
Species	No	Species	No		

Example of filled Larva Form from Tigray, Ethiopia

DATE	24/02/20	REF	b-02	LOCALI	Mae Sot
HABIT	STR	AREA	02	DIPS	12
TIME	1330	LIGHT	EX	BOTTO	M/S
TEMP	28.5	WATER	C	MOSQ	P
VEG	Grass; spirogyra;				
FAUNA	Water beetles; water skaters; fish; dragonfly larvae;				
Species	No	Species	No		
An. Minimus	25L, 02P				
Cx. Spp	02L				

Snail sampling

At the beginning of the study, water quality measurements and cross-sectional snail surveys are carried out in all surface water bodies linked to the reservoir. Monitoring can be done in a selection of 10 sampling sites during the following seasons, including the main water use points and at least 2 sites on the shore of the reservoir. Snails are sampled quantitatively using a drag scoop in deep water bodies whereas in shallow habitats 3-5 quadrates of 0.1 m² are sampled, depending on surface and morphological variation of the sites (Laamrani et al. in preparation). All material such as boulders, gravel, and floating, submerged and emerging vegetation is systematically searched for snails and snail egg masses.



Figure 2. Snail sampling by Prof. Poda in Boulbi, Burkina Faso (photo by Eline Boelee).

After identification and on-site counting, most snails are returned to their respective habitats, especially during the various rounds of monitoring. This is done so that snail populations are not influenced by the process of taking measurements. Potential intermediate host snails *Schistosoma mansoni* and *S. haematobium* (usually of the *Biomphalaria* and *Bulinus* species respectively) are taken to a simple field lab that can be set up temporarily in the village. For each of these snails, the size category is noted and they are exposed to artificial light for at least two hours to induce cercarial emergence. Phenotypic variability in the cercarial emission rhythm may modulate interpretation deduced from such observations (N’Goran et al. 1997). However, this is a time and energy-consuming method that may miss low levels of infection. Moreover, classification of species of parasites can only be done by skilled staff. Alternative procedures are now available, based on ELISA tests or DNA sequencing using PCR (Jannotti-Passos et al. 1997; Hamburger et al. 1998, 2004; Lardans & Dissous 1998). All negative snails are returned to their habitats.

Sampling adult mosquitoes

Populations of adult mosquitoes can be sampled indoors and outdoors by using CDC light traps, animal bait traps, spray catches or aspirators.

Routine collection of adult mosquitoes by light traps should be carried out in the same selection of houses once or twice a month (WHO 1975) over an extended period that covers at least two different seasons. In order to obtain a representative sample of the mosquito population, both the reservoir villages and control villages can be stratified into zones. In studies in Ethiopia, reservoir villages were divided into those near the dam, those in the middle of the village and those far from the dam. In control villages, the corresponding zones were: near potential breeding sites (water bodies or irrigated fields), in the middle of the village and away from the village.

Sampling of houses within strata can be at random, or systematic, using volunteers such as school children. Depending on the number of available light traps, collections should be made on subsequent nights. Some researchers prefer to sample the same houses two or three nights in a row.



Figure 3. Where electricity is unreliable, even for recharging, battery holders may need to be made to power the light traps (photo by Eline Boelee).

The characteristics of each house (type, absence or presence of eaves, windows, separate kitchen, and so on) are recorded and spatial coordinates are taken using a GPS. Normally the same houses are sampled throughout the study period. In each house one light trap is placed near the feet of a person sleeping under an untreated bed net. Depending on sleeping habits, another trap can be placed outside, in places sometimes used for sleeping. Light traps are operated all night from 18:00 to 07:00 hours the next day, depending on sleeping hours. Temperature, rainfall, and wind are recorded during the nights of collection. For each catch, room occupants are recorded with regard to age and gender, along with the number and species of animals found to be present indoors and outdoors close to the house. Traps that malfunction are excluded from the analyses.

For some *Anopheles* species, the biting cycle is reported to shift over time and between locations. For the identification of malaria control measures, it is useful to do repeated hourly light trap catches during main transmission seasons. The net, collection bottle, or the entire trap is then replaced every hour and mosquitoes are collected in separate labeled cups.

Outdoor-resting collections can be made in addition to light trap catches to get an indication of resting behavior. This can then be done near the same houses where light traps are operated, for several consecutive days each month. Catches are conducted in likely outdoor mosquito resting places including vegetation, animal burrows, termite mounds, large tree roots, in holes and crevices along riverbanks and near human habitations. The position of each collection site is recorded using a GPS. Collections can be made using sweeping nets, large netted frames (2 x 2 x 3m), or aspirators. Ideally all catches on all sampling occasions are carried out by the same people, and for the same length of time (for example, half an hour).

For all catches, the mosquitoes are counted and identified at the genus level, while adult anopheline mosquitoes will be identified at the species level based on morphological descriptions (Verrone 1962a, Gillies & Coetzee 1987 or other country-specific identification keys). Known vector species (identified in the literature or checked with national control programs) are classified by stages of abdominal appearance. Adult *An. gambiae* s.l. can only be identified up to species level by genotyping. If possible, sample specimens of this group are dried, stored with desiccant and then identified by polymerase chain reaction (PCR) according to Scott and colleagues (1993). If feasible, vector mosquitoes should be examined for the source of recent blood meals. In that case, the abdomens of samples of freshly fed *Anopheles* (often *gambiae*) mosquitoes are squashed individually onto Whatman No. 3 filter paper. Then the source of blood meals is determined using the direct ELISA technique adapted from Service and others (1986). The head-thorax portions of *Anopheles* spp. collected by various methods can also be used for the determination of salivary gland infection.

Role of remote sensing and GIS

Researchers are increasingly using remote sensing to detect open water bodies and measure soil humidity, which can greatly accelerate the process of estimating vector presence and risks of disease over large areas (Kristensen et al. 2001). Other tools plot disease cases into a Geographic Information System (e.g. Brooker et al. 2000; Michel et al. 2002; Klinkenberg et al.

2004). Increasingly, this technology is becoming accessible to national health services and they should be encouraged to use them.

Lessons learned

The value of these biological methods has long been established and is recognized by the health sector. They provide detailed information and primary data that may not be available for the areas being studied. At the same time they contribute to broader participatory health assessments (described in a separate tool).

Many community-led interventions for disease control target vectors. Knowledge of the location and characteristics of major breeding sites will contribute to the development of location-specific control measures. At the same time it can help the community to better understand the linkages between water management and disease transmission.

Recommendations

If national control programs exist for water-related disease in the country, it is important to collaborate with them. This also helps embed environmental disease mitigating measures for small reservoirs into regular control efforts.

Limitations of the tool

Since these studies are expensive and cumbersome, questionnaires and rapid appraisals are needed to target these studies so they can be done at the most suitable sites.

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Contacts and Links

- Eline Boelee, senior scientist water and health, <e.boelee@cgiar.org>
International Water Management Institute, PO Box 5689, Addis Ababa, Ethiopia
- Hammou Laamrani, WaDImena Project Coordinator, <hlaamrani@idrc.org.eg>
Regional Water Demand Initiative/Initiative Régionale de la Demande en Eau Internationale
Development Research Centre (IDRC) Middle East/North Africa Regional Office, PO Box
14 Orman, Giza, Cairo, Egypt
- Mekonnen Yohannes, Associate Professor, <mekonnenyohannes@yahoo.co.uk>
College of Health Science, Mekelle University, Mekelle, Tigray, Ethiopia
PO Box 1871, Private bag 1959, fax +251-344-419009
- Jean Noël Poda, DAP/ Maître de recherche, <podajnl@yahoo.fr>

Institut de Recherche en Sciences de la Sante (IRSS), Centre National de la Recherche Scientifique et Technologique (CNRST), 03 BP 7047, Ouagadougou 03, Burkina Faso

Jean-Bosco Ouédraogo, Medical parasitologist, <jbouedraogo.irss@fasonet.bf>
Institut de Recherche en Sciences de la Sante (IRSS), Centre National de la Recherche Scientifique et Technologique (CNRST), 03 BP 7047, Ouagadougou 03, Burkina Faso

Dramane Zongo, PhD student, <dramanezongo@yahoo.fr>
Université de Ouagadougou, c/o JN Poda, IRSS, 03 BP 7047, Ouagadougou 03, Burkina Faso

Links on vectors of (water-related) diseases

Integrated vector management (IVM) – directory of resources of the World Health Organization <http://www.who.int/heli/risks/vectors/vector/en/index.html>

Wikipedia on vectors [http://en.wikipedia.org/wiki/Vector_\(biology\)](http://en.wikipedia.org/wiki/Vector_(biology))

Research at NRI on vector-borne diseases of humans <http://www.nri.org/research/vectors.html>

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Small Reservoirs Tool Kit

Master Reservoir Sheet as prepared for Sri Lanka (Amerasinghe 2001, unpublished)

TANK DATA (separate sheet for each tank)														
Tank = code for particular tank														
Tnkspl = tank spill (response: Yes/No)														
TnkLvl(m) = water level at outlet (measure in metres)														
TnkAr(%) = area under water as % of max tank water area (eye estimation)														
TnkPl = tank pools (present/absent)														
TnkWdth(m) = width of margin area with pools (measure or estimate by eye in meters)														
TnkPlTyp = tank pool type (define types: e.g.: hoof print, borrow pit, etc)														
TnkPlNo = Number of Tank pools (count by eye or binoculars)														
VgWtr(%) = % water area covered by surface vegetation (estimate by eye)														
VgCvr(%) = % Veg. Cover of water margin as % of total margin (estimate by eye)														
WQDeg = water qual. (subjective estimation: clear / turbid / foul)														
KtSpAr(m) = Width of seepage area below dam (measure or estimate by eye in meters)														
KtVgCvr(%) = Degree of seepage area covered by standing/surface veg. (estimate by eye, as a percentage)														
TbVg(%) = Degree of veg. on tank bund														
<i>Tank</i>	<i>Date</i>	<i>TnkSpl</i>	<i>TnkLvl (m)</i>	<i>TnkAr (%)</i>	<i>TnkPl</i>	<i>TnkWdth (m)</i>	<i>TnkPlTyp</i>	<i>TnkPlNo</i>	<i>VgWtr (%)</i>	<i>VgCvr (%)</i>	<i>WQDeg</i>	<i>KtSpAr(m)</i>	<i>KtVgCvr (%)</i>	<i>TbVg (%)</i>

Small Reservoirs Tool Kit

Example of Larva Sheet as filled in during Small Reservoirs (locally called “tank”) study in Sri Lanka (Amerasinghe 2001, unpublished)

Habitat = code for specific habitat type (seepage pool, tank bed pool, canal, hoof print, tank margin, etc etc)

Location = specific small tank name or locality name that serves as the major identifier

Area = area (m²) of water surface of specific habitat sampled for mosquito immatures (at the rate of 6 dips per m²)

Water = quality of water (clear [C], turbid C[T], foul [F]) determined by appearance and smell

Light = Condition of light at specific habitat sampled (fully exposed to sun [EX], partially exposed to sun [ES], shaded [SH])

Bottom = condition of specific habitat bottom (MUD, SAND, ROCK or combinations thereof)

Temp = temperature (°C)

Mosquito = mosquito positive [+] or negative [-]

Veget1 = name of dominant vegetation type in sampled specific habitat (e.g., DEAD LEAVES, ALGAE, SPIROGYRA, CABOMBA etc depending on plants). Leave blank if nothing

Veget2 = name of next dominant vegetation type in sampled specific habitat (e.g., DEAD LEAVES, ALGAE, SPIROGYRA, CABOMBA etc depending on plants). Leave blank if nothing

Veget3 = name of next next dominant vegetation type in sampled specific habitat (e.g., DEAD LEAVES, ALGAE, SPIROGYRA, CABOMBA etc depending on plants). Leave blank if nothing (*column deleted from this example*)

Fauna1 = dominant associated fauna in habitat. Leave blank if nothing

Fauna2 = next dominant associated fauna in habitat. Leave blank if nothing

Fauna3 = next next dominant associated fauna in habitat. Leave blank if nothing (*column deleted from this example*)

Mosq.Spp = Abbreviation for mosquito species found in habitat (please note: make multiple records if 2 or more mosquito species in same sample)

LV = larvae

PU = pupae

Immdip = immatures per dip (easy to compute from Area because we always take 6 dips per m² sampled)

**** Simply add more columns if additional parameters are measured (e.g., water quality parameters like EC, pH etc)**

DATE	REF	DATEREF	HABITAT	LOCATION	AREA	WATER	LIGHT	BOTTOM	TEMP	MOSQ	VEGET1	VEGET2	FAUNA1	FAUNA2	MOSQ. SPP.	LV	PU	IMMDIP
02-10-95	18	18/100295	TNK	HMW	5.00	C	EX	MUD	35.00	+	ALGAE		FROGS		ANMA	1	0	0.03
02-10-95	18	18/100295	TNK	HMW	5.00	C	EX	MUD	35.00	+	ALGAE		FROGS		ANSU	1	0	0.03
02-10-95	19	19/100295	TNK	HMW	5.00	C	EX	MUD	35.00	-	ALGAE		FROGS			0	0	0
02-10-95	20	20/100295	TNK	HMW	5.00	C	EX	MUD	34.00	+	LOTUS		FROGS		CX.SPP	3	0	0.1
02-10-95	21	21/100295	TBP	HMW	5.00	C	EX	MUD	35.00	+	LOTUS		FROGS		CX.SPP	7	0	0.23
02-10-95	22	22/100295	TBP	HMW	5.00	C	EX	MUD	36.00	-	ALGAE					0	0	0
02-10-95	24	24/100295	TBP	HMW	5.00	C	EX	MUD	35.00	+					ANJA	1	0	0.03
02-10-95	25	25/100295	TBP	HMW	5.00	C	EX	MUD	35.00	-	ALGAE	LOTUS	FROGS			0	0	0