3. SAMPLING LARVAE & PUPAE

PRINCIPLES

- Surveillance by larval survey is of key importance as all mosquitoes rely on water bodies for their development, thus allowing a focused and rapid inspection (sampling and identification can be performed on the same day) and offering usually a good cost-benefit ratio
- Besides, larval sampling is of particular interest with mosquito species whose females are not or only rarely attracted to the commonly used trap systems

LIMITATIONS

- There are great differences between the kinds and specific characteristics of colonised water bodies (e.g. size, water quality, natural or man-made) and the ecological surroundings they are embedded in (e.g. rural, urban, nature)
- Also, larvae of univoltine species can only be detected seasonally while those of multivoltine species often occur throughout the whole vegetation period
- Larval surveys therefore require a detailed knowledge about mosquito ecology

WHERE & WHEN

- The best season for larval sampling depends on the latitude and altitude, as well as on the mosquito species; In general, mosquito larvae develop when water temperatures are above 10 °C
- Six main classes of larval habitats can be distinguished:
  - **Stagnant temporary water bodies** (ditches, ponds, forest ditches, fens, flooded meadows or forest)
  - **Running waters** (rivers, streams, ditches, drains)
  - **(Semi-)permanent water bodies with vegetation** (ponds with vegetation, marshes, canals)
  - **Semi-natural water bodies without vegetation** (puddles, road tracks, new ditches, etc.)
  - **Natural containers** (tree holes, rock pools, etc.)
  - **Man-made (artificial) containers** (e.g. pots, catch basins, pits)

WHERE & WHEN

- In natural areas, larvae can be found in puddles, road tracks, swamp areas, drains, ditches, irrigated croplands, streams, riverbeds, ponds, tree holes etc. but also in human-made containers such as drinking troughs, discarded waste containers, tyres, tarpaulins, etc.
- In urbanised areas, larval sampling must focus on the available human-made water bodies found both in private and public environments, below and above ground level, such as discarded containers, flower vases, flower pot dishes in gardens and cemeteries, used tyres left outdoors, rain water barrels, road drains and catch basins, pits, etc.

Stagnant temporary water bodies

Flooded coastal marsh


Wetlands

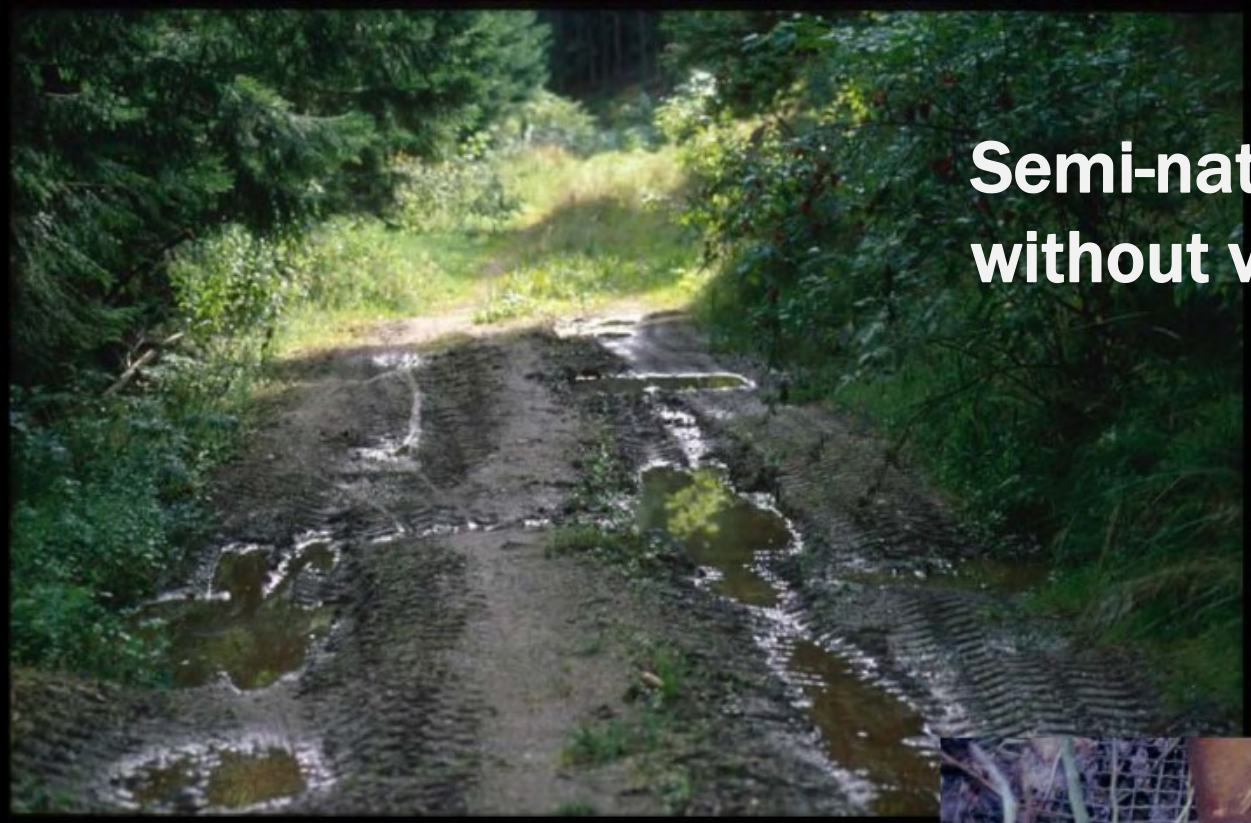
(Semi-)permanent water bodies with vegetation

Spring

Canal

Running waters

Semi-natural water bodies without vegetation



Puddles and ruts

Well

Natural containers

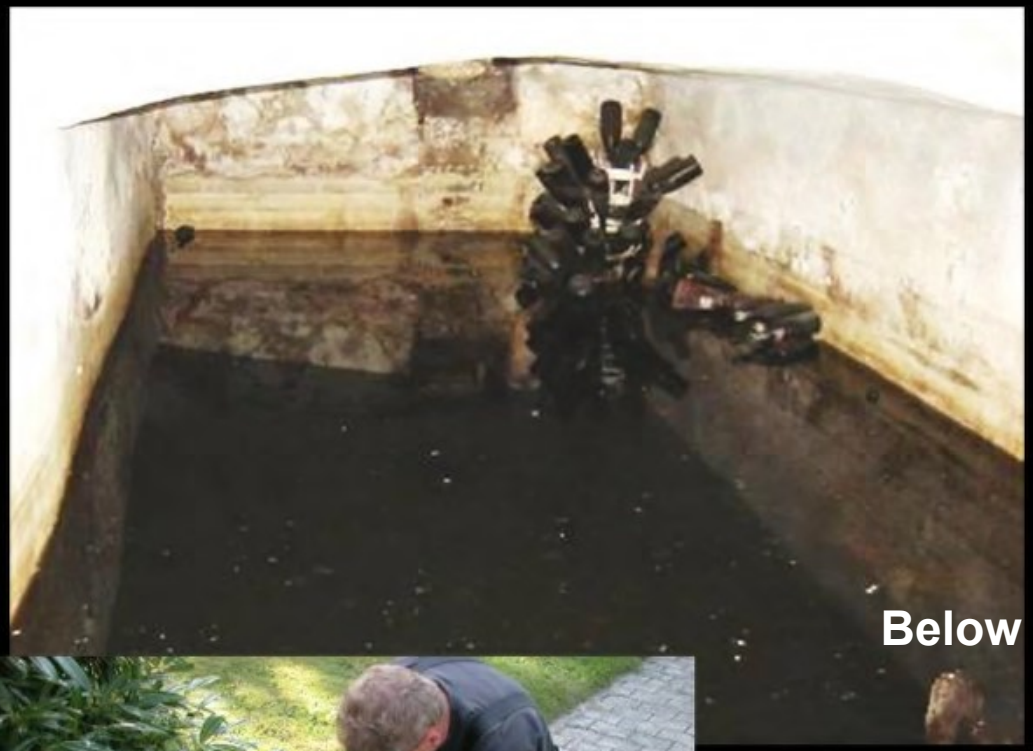
Mineral: rock pools

Salted water

Natural containers

Vegetal: phytotelms


Man-made (artificial) containers



Below ground

Above ground

Diversity and specificities in larval habitats

METHODS & TOOLS

Once the sampling area is defined, possible breeding sites have to be identified:

- On Google or Bing satellite imagery: identification of wetlands, swamps, pools, ditches, etc.
- On place: identification of all kinds of small natural and artificial water bodies

METHODS & TOOLS

- Depending on the size and design of their breeding sites, mosquito larvae can be collected by netting, dipping or sucking
- Larger water bodies can be sampled by classic dippers or plastic trays, or by fine-meshed (≤ 0.5 mm) aquatic nets (e.g. aquarium water nets) and sieve
- Smaller water bodies (e.g. tree holes) can be checked for the presence of larvae by dipping with a ladle or by aspirating water with a tube or a pipette
- Collected water can be inspected better for the presence of juveniles when decanted in a white plastic tray/bowl

STANDARD DIPPING METHOD

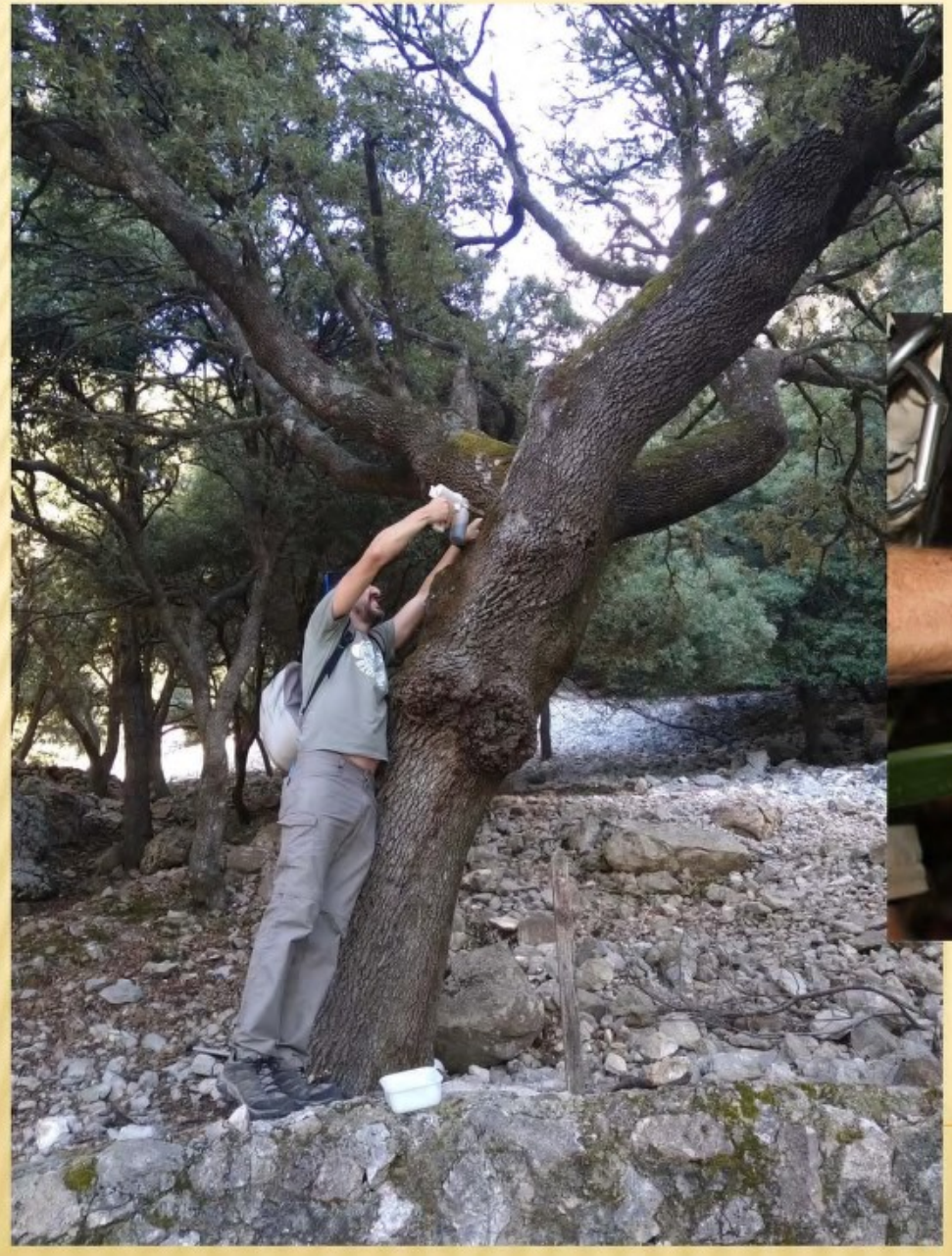
- Dippers can be used as a survey tool for determining the abundance of larvae by taking various samples from designated sites in the habitat of interest, and then counting the larvae of each dip, whereas netting, which allows to sample over larger parts of the habitats, is more appropriate to determine presence/absence
- The netting/dipping method will vary with water depth, presence of aquatic vegetation or debris, and water clarity; Five to 20 sweeps/dips are recommended, depending on the site size and variety of micro habitats (e.g. with or without vegetation, floating or erected vegetation, different water depths, shaded or sun-exposed)
- Several dipping techniques exist, with differing efficiency to collect the various mosquito genera; For routine immature monitoring of all species to compare across a range of aquatic habitats without genus-specific approach, complete or partial submersion is recommended, depending upon how deep the water is

DIPPING

- Dipping methods

Dipping method	Targeted mosquito genera	Method details	Notes
Shallow skim	*Anopheles*	The leading edge of the dipper is submerged at approximately 45° and about 2.5 cm below the water surface. The dipper is drawn along the water surface and filled at the end of the stroke.	The method works better for *Anopheles* larvae that remain at the water surface comparatively longer than do other mosquito larvae after the dipper enters the water. A good technique of sampling when submerged macrophytes have leaves just below the water surface.
Complete submersion	*Aedes, Ochlerotatus,* (*Culex, Culiseta*)	The dipper is submerged quickly in open water, usually in floodwater habitats. The dipper is brought up to the water surface through the submerging larvae that have reacted to the disturbance created by submerging the dipper.	This method is used primarily to sample mosquitoes whose larvae respond rapidly to the dipper entering the water, but are visible. It is also appropriate for sampling larvae adjacent to vegetation. The dipper is brought to the water surface while contacting the emergent vegetation.
Partial submersion	*Anopheles, Culex, Culiseta*	The dipper is submerged approx. 45° along the emergent vegetation. Water flows rapidly into the dipper. The dipper is not moved horizontally. but can be moved vertically to scrape along the edge of the emergent vegetation.	The method works well when sampling in robust emergent vegetation such as cattail and bulrush. The suction created by water flow into the dipper and scraping also collects small insect predators and herbivores associated with mosquito larvae on or near the vegetation.
Flow-in	*Aedes, Ochlerotatus,* (*Culex*)	This technique is used in shallow water that has a depth < height of the ladle of the dipper. The bottom of the dipper is pushed into the substrate and the water with associated larvae and debris flow into the dipper.	This method works well in shallow habitats, root masses and other habitats that are shallower than the dipper's profile.
Scraping	*Coquillettidia*	The dipper is scraped against the underside of floating vegetation to dislodge attached larvae. The scraping action is usually a vigorous back-and-forth motion.	Used to sample larvae that reside under and usually attached to floating vegetation or the roots of floating plants. Because a vigorous back-and-forth motion is used with the dipper completely submerged, this technique works best with dippers having a screened bottom.
Simple ladle	*Culex*	A quick flip of the wrist is used to submerge completely the dipper just below the water surface. The technique is similar to taking water to drink.	Not a preferred method, especially if the sample is not taken adjacent to a mosquito microhabitat. This technique would be adequate in hypereutrophic situations where the abundance of larvae often approaches 1000/dip.
Background	*Aedes, Ochlerotatus*	The dipper is sued to provide a light background against which darker coloured immature mosquitoes are more easily seen. After mosquitoes are found, they are collected by quickly pulling the dipper through the water surface.	A technique used primarily to identify mosquitoes inhabiting woodland ponds and pools.

METHODS & TOOLS




RES-Q VAC

SAMPLE HANDLING

- The larvae should be first transferred with a pipette to a small cup or bowl with fresh clean water as a washing procedure
- If much debris or sediment is still present, additional serial transfers should be made until elimination of suspended particles has taken place
- As much water as possible should be removed from the cup or bowl using the pipette
- In the lab, water heated to about 60°C can be poured into the cup or bowl
- In the field, the larvae can be first transferred to a vial using the pipette after which pure ethanol can be added
- As soon as the larvae float up to the surface, the liquid is removed with a pipette and replaced with a quantity of 70-80% ethanol
- After 5 min, the larvae are transferred with a pipette (do not use forceps!) to a vial with 70-80% ethanol
- No more than 20 larvae should be placed in a single vial as the water contained in the bodies of the larvae will significantly dilute the concentration of a small quantity of ethanol and jeopardise preservation

SAMPLE CONSERVATION

- For immediate morphological or genetic ID: in vials with 70-80% ethanol
- For morphological ID only after further development: in vials/small containers together with water of their breeding place for rearing L1-L3 larvae to L4 larvae (which can be identified with higher reliability) or for keeping the larvae until adult emergence
- Pupae: to keep until emergence of adults

FIELD DATA AND PARAMETERS TO BE RECORDED

- ☑ Place (georeferenced)
- ☑ Environment/land use
- ☑ Type/category of site inspected
- ☑ Short description of site including size and colour of water
- ☑ Date, time
- ☑ Number of dips/sweeps
- ☑ Results (number of larvae)

CHECKLIST FOR THE FIELD STUDY

- ☑ Smartphone or field data reporting system (e.g. VECMAP™ app)
- ☑ Dipper and/or white plastic tray
- ☑ Fine-mesh aquatic net
- ☑ Fine-mesh sieve
- ☑ Kitchen ladle
- ☑ Tubes
- ☑ Pipettes
- ☑ Vials
- ☑ Ethanol
- ☑ Labels
- ☑ Pencil
- ☑ Field magnifying glass

DATA REPORT AND MANAGEMENT

- Study protocol
- Field form or Smartphone App
- Data base
- Sample identification
- Analysis

FICHE de PRÉLÈVEMENT – INVENTAIRE et BIOLOGIE des CULICIDES

Partie grisée à remplir obligatoirement

Observation Culicides

N° Fiche : Loc. / An. / N°

Date / heure : jj / mm / aa / hh / mn

N° Gîte : Code gîte (à défaut : remplir cadre Obs. Gîte)

Auteurs : Récolte / Détermination

FAUNE COMPAGNE (dominance et particularités)

Microfaune	Ab.	Prédateurs	Ab.	Autres	Ab.

CULICIDES

Larves	%	Nb/stade	Nb/sexe	%	Imagos

Observation Gîte

LOCALISATION

Pays / Région/Province / Commune / Code Insee

Région naturelle / Lieu-dit / Station / Altitude ____ m

Coordonnées géogr. UTM WGS 84 (GPS) : Lat. / Long.

ou Lambert II étendu : Lat. / Long.

ou Google Earth : Lat. / Long.

N° cartes : 1:100 000e / 1:25 000e / autre

DESCRIPTION DU GÎTE

Hauteur // sol ____ cm

Profondeur // sol ____ cm

Surface eau maxi ____ m²

FLORE (niveau écologique / dominance et compagnes)

Pleine eau	Ab.	Berge	Ab.	Couvert	Ab.

Méthode de piégeage

- ☐ bac
- ☐ filet troubleau
- ☐ filet fauchoir
- ☐ piège CO_2
- ☐ piqueur
- ☐ piqueur mollet
- ☐ p. m. standard
- ☐ sous abri
- ☐ substrat (œufs)

Météo (capture d'adultes)

T° ____ °C Hygro. ____ %HR

Pression ____ mm Hg

Coucher de soleil ____ h ____ min

Ciel : ☐ clair ☐ voilé ☐ nuageux ☐ couvert ☐ orageux

Vent : ☐ nul ☐ très faible ☐ faible ☐ moyen ☐ fort

Hydrologie (prélèvements larvaires)

Densité larvaire ____ larves/litre

H. eau ____ cm

Surface ____ m²

Recouvrement végétal ____ %

Couvert (végétal ou artificiel) ____ %

[Cl-] ____ mg/l DBO ____

T° ____ °C pH ____

Mise en eau du gîte : ☐ pluies ☐ nappe ☐ ruissellement ☐ drainage ☐ suintement ☐ source ☐ artificielle ☐ déb^{lt} rivière ☐ déb^{lt} étang ☐ déb^{lt} mer

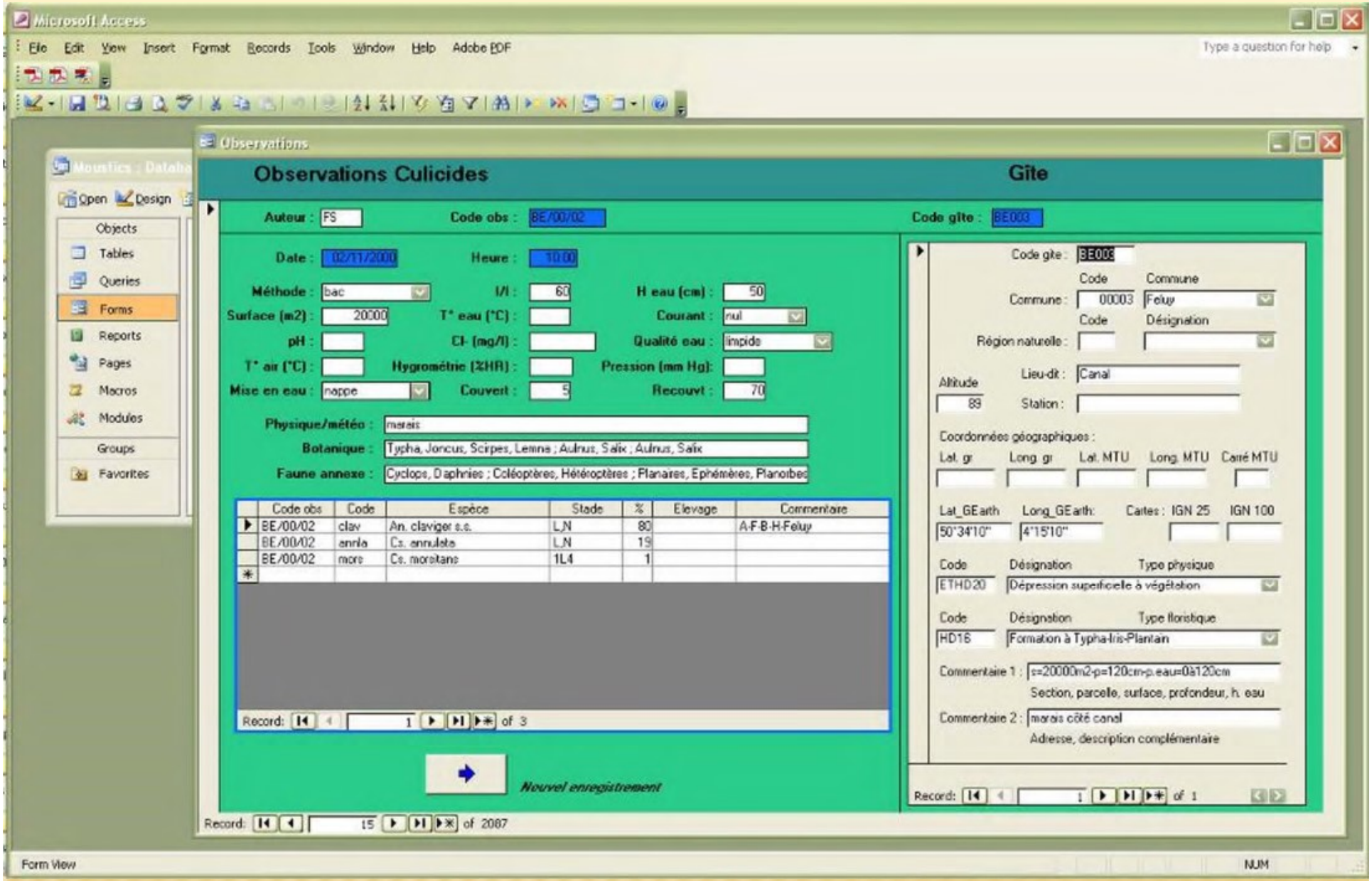
Courant : ☐ nul ☐ très faible ☐ faible ☐ moyen ☐ fort

Qualité eau : ☐ limpide ☐ trouble ☐ verdâtre ☐ jaunâtre ☐ brunâtre ☐ noire ☐ putride (m.o.) ☐ ferrugineuse

Commentaires

Flacon de transport n°

Example of field form



Example of data base (entry form)

SAMPLE IDENTIFICATION (LAB)

STANDARDISING FIELD PROTOCOLS

EXTERNAL SCIENTIFIC REPORT VectorNet European network for sharing data on geographic distribution of arthropod vectors, transmitting human and animal disease agents

APPROVED: 15 June 2018

doi:10.2903/sp.efsa.2018.EN-1435

Field sampling methods for mosquitoes, sandflies, biting midges and ticks

VectorNet project 2014-2018

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Abstract

ECDC and EFSA have the mandate to assess the risk of infectious diseases affecting pul
health and strengthen the capacity for their prevention and control in the EU. Vector-b
as a specific group of a (re-)emerging infections, pose a threat to European public heal
particular attention. One important aspect of preparedness for vector-borne diseases is
or surveillance of the introduction, establishment and spread of the main disease vecto
surveillance or monitoring campaign starts with the development of a well-consid
strategy. Depending on the target species, a range of sampling methods is available. Th

https://ecdc.europa.eu
https://efsa.europa.eu

Sampling protocols
→ larvae
→ adults
→ presence/absence
→ towards standardisation

Catching methods

- **Net sweeping.** Rustle the vegetation with a stick in one hand; with the other hand, sweep the space above the vegetation with a hand-net; quickly remove all caught mosquitoes from the net, using a mouth or mechanical aspirator to avoid the loss of scales from the integument of the mosquito.
- **Aspirating in shelters.** Illuminate the wall with an electric torch and catch adults resting on/hovering above the walls (usually in shaded and humid places) with a small mechanical aspirator (or directly with a mouth aspirator).
- **Aspirating in tree holes, rock crevices, small hiding places.** Catch hovering adults (usually in shaded and humid places) with a small mechanical aspirator, or, if available, a backpack mechanical aspirator.

Specific guidelines for sample collection

Where to search?

Bedrooms, bunkers, animal sheds, caves, (semi-)underground cellars (including wine cellars, mushroom-rowing cellars, etc.), vegetation (grass at breeding sites, tree holes and piled-up branches in the surrounding); at favourable places.

What time of year?

Collections can be performed during the entire year; during the cold period of the year, mosquitos can be sampled in overwintering places (e.g. caves, bunkers, animal shelters, (semi-)underground cellars, tree holes); during mosquito season, all places listed above can be surveyed.

What time of day?

Collection should preferably take place early in the morning during the mosquito season; any time of day is fine for winter sampling.

For how long?

- Duration: 30 minutes at each site.
- Abundance data: quantitative data are related to the intensity of the sampling effort (total number of mosquitoes collected in 30 minutes per collector per site) or to the number of sites examined.

Sampling methods: larvae

Trapping methods

Six main classes of larval habitats can be distinguished:

- Stagnant temporary water bodies (ditches, ponds, forest ditches, fens, flooded meadows or forests)
- Running waters (rivers, streams, ditches, drains)
- (Semi-)permanent water bodies with vegetation (ponds with vegetation, marshes, canals)
- Semi-natural water bodies without vegetation (e.g. puddles, road tracks, new ditches)
- Natural containers (e.g. tree holes, rock pools)
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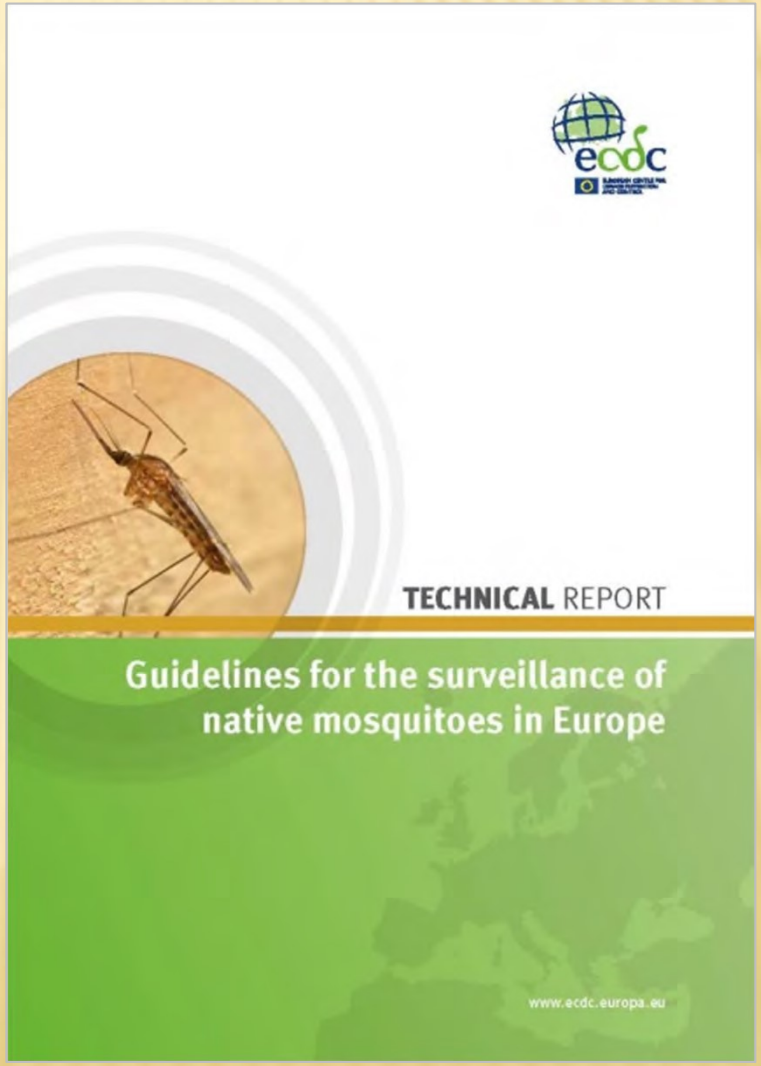
ECDC GUIDELINES FOR THE SURVEILLANCE OF MOSQUITO VECTORS IN EUROPE

https://ecdc.europa.eu



SUPPLIERS FOR FIELD EQUIPMENT

https://eu.biogents.com/mosquito-traps/ (Germany)

https://www.bioquip.com/ (USA)

https://www.bioquip.com/ (USA)