## → Basic equipment needed to set up your base camp:

- Hammock with mosquito nest (or robust lightweight tent if necessary).
- Large heavy-duty tarps (size will depend on the number of researchers staying in the camp).
- Plenty of solid rope to attach your hammock and fix the tarps.
- Heavy-duty tape to repair potential tears in the tarp.
- Light sleeping bag (nights may be surprisingly cold in the forest).
- Light pillow.
- Machete + file.
- Light foldable seat (an optional luxury).



Fig. 19. Base camps. A. Basic base camp for short-time stays; B-C. Solid base camps for longer stays, note the separated "field lab" on photograph C (front); D. Tents on the summit of a tepui, note solar panels and 12 volts battery to provide electric power. (Photos by P. J. R. Kok).

## **Energy solutions**

We use rechargeable batteries, which are charged through a 12 volts solar battery alimented by two solar panels (Fig. 20). All of our electronic and electric equipment (laptop, satellite phone, recorder, headlamps, etc.) runs thanks to solar power. Rechargeable LR6 (AA) NiMH batteries (used in headlamps, DAT recorder, etc.) are charged with a 15 minutes charger. If you plan to run a laptop, solar panel(s) with a minimum power output of 52 watts is recommended. We chose this solution mainly for ecological reasons and for minimizing our impact

on the environment (no dead batteries, no fuel to run a polluting generating unit). Note that this technology evolves very quickly and many new excellent products can be found on the market. Modern solar panels are very light, foldable, and charge even in cloudy conditions. Some new batteries include an inverter, are very small and lightweight. We have a preference for *Brunton*® products and use two *Solaris*® 26 foldable solar panels.

## → Basic equipment needed to provide electric power:

- Foldable solar panel(s), minimum power output of 52 watts recommended.
- Inverter: necessary to operate your electronic/electric devices.
- Solar controller: prevents overcharging the 12 volts battery and safely permits the battery to remain in constant charging.
- 12 volts solar battery, preferably dry cell.



Fig. 20. Electric power provided by solar panels, here charging the emergency satellite phone. See text for details. (Photo by P. J. R. Kok).

## Food and cooking

Adequate food is essential during field research. Do not forget that you will probably walk long distances, sleep little and work hard. We believe that food should always be bought in the country where the research is done, avoiding expensive excess baggage costs, contribute to the local economy instead!

You will need to find a clever compromise between weight and calorific value. We usually take cereal bars, oatmeal instant packs (there are different flavours), cereals, raisins, coffee/tea, sugar cane and dehydrated milk for breakfast; Chinese noodles instant packs, rice, cassava farine, dehydrated soya (called "chunk" in Guyana), onions, garlic, and hot sauce for other meals. When in the field, we usually only eat twice a day, once in early morning and once in late afternoon.

Lighting a wood fire can be somewhat tricky in some wet places and it may be risky to rely on it to cook. Furthermore cooking on wood fire is often time-consuming and maintaining the fire requires time and attention. We prefer to use a liquid-fuel stove and we have a fondness for the *Dragonfly* from *MSR*<sup>®</sup>, which is lightweight and burns many different fuels (white gas, kerosene, unleaded auto fuel, diesel, and jet fuel). Only a few minutes are needed to boil water and the stove is very fuel-efficient.

## → Basic equipment needed for cooking and eating:

- Multifuel stove and accessories.
- Set of lightweight cookware.
- Robust cups, knifes, and spoons.
- Weatherproof lighter(s).
- Small fuel container.

## Water

In tropical rainforests water is everywhere and is usually not a problem. It is recommended that all water be sterilized, especially near local communities, as there might not be a distinction between washing and drinking water. We usually do not disinfect water when travelling in remote areas, but it is always better to do it (gastric problems can ruin your expedition). You can obtain safe water by adding water purification tablets (*e.g. Micropur*). However, we prefer to use Ultraviolet disinfection (*SteriPEN*®) because it is much faster (about 50 seconds for 0.5 litres) and does not give a bad taste to water. You also can collect rainwater using a tarp and adequate containers. We always take an inflatable water container to stock water at the base camp.

## Hygiene and clothing

Good hygiene is important in the field, especially to avoid skin problems. Avoid walking barefoot near some local communities, especially those in sandy areas as you have a high chance of collecting sand fleas (*Tunga penetrans*, also called "chiggers"). These parasites may be very painful and must be carefully extracted. Regularly check your body and remove ticks and other parasites as soon as possible as some carry diseases.

Use lightweight clothes that dry quickly, wear long rubber boots in the field (do not forget good socks), and slippers in the camp. Always try to keep a set of dry clothes and use small waterproof bags to pack them. Do not forget a robust rain cap.

#### Pharmacy and safety equipment

During field research you will probably be out of reach of immediate medical aid, as such some basic safety equipment and drugs from a good pharmacy, and some common sense precautions are thus necessary.

Covering all health hazards is beyond the scope of this manual; you should always carefully check with your doctor for the recommended vaccination and the appropriate medication to carry with you in the field. When possible, use orodispersible tablets as you can take them while walking or if clean water is not immediately available.

Malaria is rampant in many tropical countries and, if contracted, oftentimes kills both residents from local communities and researchers. An adequate malaria prophylaxy is mandatory. Note that antimalarial tablets are often much less expensive in tropical countries.

Leaving your pharmacy to local communities once the field trip is completed is an excellent idea, but this is only valuable if you explain drugs indication and dosage!

## → Basic field first-aid kit:

- Plenty insect repellents.
- Malaria prophylaxy.
- Thermometer.
- Skin suture set.
- Syringes and needles.
- Topical anaesthetics.
- Disposable scalpels.
- Sterile skin closure strips, several sizes (Steri-Strip™).
- Tourniquet.
- Sterile compresses.
- Sterile plasters, including blister plasters (Compeed®).
- Bactericide aqueous disinfectant.
- Pain tablets (avoid aspirin if there is a malaria risk).
- Allergy relief/allergy symptoms medicine.
- Epinephrine.
- Broad-spectrum antibiotics.
- Antibiotic cream for skin/eyes.
- Antifungal cream.
- Flu medicine.
- Anti-inflammatory cream.
- Elastic bandages.
- Anti-diarrhoea medication/adsorbing preparations.
- Intestinal amoebiasis treatment.
- Non-steroidal anti-inflammatory (also exist in very useful patches).
- Sun blocker.
- Sleeping tablets.
- Tweezers.

## → Basic safety equipment:

- Venom suction pump.
- Survival blanket.
- Satellite phone (optional).

# 3.3. Specimens and data collection

For further detailed information, we strongly suggest the reader to refer to Heyer *et al.* (1994), which is the most important reference available for researchers interested in measuring and monitoring biological diversity in amphibians. Also see Simmons (2002).

## 3.3.1. Basic collecting equipment

In order to collect specimens and data you will need some basic equipment (additional specific equipment is provided below, under specific sections).

As most amphibians are nocturnal, a good headlamp is probably one of the most important devices you will need in the field: at night frogs and toads may be spotted by their bright red eyeshine, which is the reflective effect of the *tapetum lucidum*, a reflecting layer found behind the retina that improves vision in low light conditions. We use the *Duo Led 5* from *Petzl*® (with rechargeable batteries, see "Energy solutions" above), which is waterproof down to -5 meters and allows two kinds of lighting: halogen for focused lighting (up to 100m) and LED's for wide, proximity lighting. The *Petzl*® *Myo XP* is lighter and is an excellent alternative.

Most of the time, amphibians are captured by hand or with a small aquarium net, quickly slamming the net over them.

It sometimes happens that a member of the collecting team detects a frog at a certain distance (by eyeshine detection for instance) that other field investigators cannot locate. While walking through dense vegetation it may be difficult to stay focused on the animal and the specimen may be lost. To circumvent that problem, a member of the team can use a small laser pointer to indicate the frog's position while another investigator goes to collect it.

To avoid getting lost take some colour flagging tape to mark the trails, which will help you find your way back.

For note taking at nights in the camp, we use a small dynamo headlamp (rarely candles) in order to save batteries. If you have a well-charged 12 volts battery, an economic bulb in the camp is helpful.

After capture, medium to large specimens are placed in plastic bags (ziplock bags are quite effective), and small or tiny specimens are transferred in screw-top small containers (urine sample containers are ideal). If possible the field tag should be immediately placed with the associated specimen (see "Field notes and labels" below).

A global positioning system (GPS) is a must that will allow you to record the exact geographic coordinates of your base camps and collecting localities. We use the 60CSx model from *Garmin*®, which also records altitude.

You will also need some measuring devices to record environmental and specimen data. A thermometer and a hygrometer are the basics, a pHmeter will be useful to record acidity of water in which tadpoles are found. We use callipers for measuring small specimens and measuring tape for larger individuals. Measuring tape is also used to record, for example, distances between animals and water, or distance between the animal and the ground. Spring scales are used to weigh specimens.

#### → Basic equipment needed for collecting specimens and data:

- Headlamp, batteries and spare bulbs.
- Dynamo headlamp, candles, or economic bulb to take notes at night.
- Small aquarium net.
- Airtight plastic bags and small containers.
- Shoulder bag to carry the collected specimens.
- Global Positioning System (GPS).
- Colour flagging tape for trail marking.
- Thermometer/Hygrometer, pHmeter (optional).
- Callipers.
- Measuring tape.
- Spring scales (10, 100, and 500 grams are sufficient for amphibians).
- Hand lens.
- Binoculars.
- Machete and knife + file.

## 3.3.2. Number of voucher specimens required

The number of specimens required to establish identification is variable from one species to another and it is impossible to generalize. Whenever possible, we recommend the minimum of 10 adult voucher specimens from each site; ideally 25 adult specimens should be collected. It is recommended to collect both sexes, juveniles, and larvae. The minimum number of collected larvae should be 20, preferably including different stages of development (see "Sampling of amphibian larvae" for further details).

The number of specimens collected will, of course, depend on the rarity of the species and/or the difficulty to collect species representatives (*e.g.* in case of highly arboreal species or fossorial species). It is usual that even during an extensive survey you will not encounter more than one specimen of a peculiar taxon.

Preparing specimens is time-consuming and you should never collect more specimens than you will be able to handle properly. Fewer well-prepared specimens associated with accurate data are always better than many specimens in poor state of preservation and lacking pertinent information. Note that collection of rare species may endanger the population: in case of known endangered species or very rare taxa, fewer vouchers should be collected, but at least a single representative should be retained.

It should be mentioned that some species considered as common are curiously poorly represented in museum collections, which for example precludes exhaustive study of intraspecific variation. Thus do not refrain to collect good samples of so-called "common species".

#### 3.3.3. Field notes and labels

It is crucial that each collected specimen be associated with detailed relevant data. In order to do so, a numbered label (tag) is securely attached to each specimen. Tags should be made of solid paper instead of any hard material (like metal or plastic) that could damage the specimen during transport. Indelible ink or tags with perforated numbers (Fig. 21A) should be used. The use of coloured tags or coloured inks must be avoided as they might discolour the specimens.

In frogs the tag should be attached around the knee (Fig. 21C), or around the waist in very small specimens (Fig. 21D). In caecilians, the tag is attached around the neck or around midbody. The best knot to attach the tag, avoiding that it unties during transport, is probably the surgical double knot (Fig. 21B); make two to be sure. Our numbered tags always include initials of the main investigator (Fig. 21A).

Series of tadpoles, or other very small samples collected together, are preserved separately in screw-top vials (see below) and are kept in a small leakproof plastic bag (we use *Whirlpak*® from *Nasco*), in which the tag is inserted (Fig. 22).

The tag number will be retranscribed in the field book and associated with temporary field identification and detailed data about the collected specimen.

Minimum data associated with the collected specimens are: precise locality (if possible geographic coordinates, which are referenced to map datum WGS84), elevation, date and time of collection, collector's name, sampling/detection method, general habitat, microhabitat, type of activity before capture and basic weather data (see Fig. 23). It might be difficult to take extensive notes while collecting in the field, especially at night or during heavy rains. An interesting alternative is to use a mini voice recorder to record the data on a tape (digital ones are very small and record on a hard drive) and subsequently report the data in your field book. These recordings are also great back-up solutions.

Field books should be made of solid, all-weather, waterproof material, and waterproof inks must be used (we recommend *Rite in the Rain*® products, Fig. 23). We always use paper with metric grid, which is useful when taking photographs (see below). Pencils are suitable and cheaper alternative to all-weather pens. All data should be saved as soon as possible in electronic format (*e.g.* on a CD or external hard drive) in case you lose or damage your field book. Keep in mind that your field book itself is as valuable as your voucher specimens.



**Fig. 21.** Tagging voucher specimens. A. Two different types of tags that can be used in the field: printed tag with indelible black ink (upper), and tag with perforated number (lower); B. How to tie a surgical double knot; C. In frogs, tag should be attached around the knee; D. In very small specimens tag should be attached around the waist. (Photos by P. J. R. Kok).



**Fig. 22.** Series of tadpoles (like illustrated here) and other small samples are packed in small leakproof plastic bags in which the field tag is inserted. (Photo by P. J. R. Kok).

P.J. R.KOK KNP 03/06 PK 1282 Hypriboos M.Sp. Koreten National Bark, Pyons Between Boy Jeant View Johnson View 5º 6'51"N 53º 28'57"W con 400 m elevation Celle ched 13/03/ 2006 - 8= 30 PT or colling from the phytokelin of Brocchimis inice the Edges between ford and so Clear sty 7º 23°C Recorded on TARE 1 12:08500 -> 10:15 50

Fig. 23. *Rite in the Rain*® field book and basic notes about a voucher specimen. (Photo by P. J. R. Kok).

➔ Basic equipment needed for labelling your specimens and recording your data:

- Field tags (to be sure, take *ca.* 1000 tags for a three weeks long fieldtrip).
- String to attach field tags to specimens.
- Small scissors to cut strings and tags.
- Small forceps.
- All-weather notebook(s).
- All-weather pen(s) and/or pencil(s).
- Digital mini voice recorder (optional).
- Laptop and external hard drive to back-up your data (optional).

## 3.3.4. Photography of voucher specimens and habitats

Most amphibians quickly lose their colour in preservative. Sometimes colours may drastically change (*e.g.* the bright green *Phyllomedusa bicolor* becomes purple in preservative, the green *Hypsiboas cinerascens* fades to white). Good photographs of preserved specimens in life are invaluable. They will facilitate the description of colours and patterns, and zooming in the digital picture will help you to distinguish some features that often disappear in preservative (folds, texture of skin, etc.). You will sometimes be surprised to see some details that were completely overlooked in the field.

Photographs should show features used for identification. We suggest taking at least a dorsolateral and a ventral view of each specimen (ventral view on paper with metric grid if possible). We usually take much more photographs of each individual, from different angles, including details of peculiar patterns and/or morphological characters.

Photographs of tadpoles are also very valuable. We usually take photographs of larvae in a Petri dish deposited on a paper with metric grid (the same paper used in our field book, Fig. 24).



Fig. 24. Taking photographs of tadpoles is invaluable, notably to record their colour in life. (Photo by P. J. R. Kok).

When possible, photographs should be taken *in situ*, but this is rarely achievable. We recommend the use of a small tent with a large front opening (Fig. 25A) in which you will reconstitute the microhabitat of the animal. In case the animal tries to escape (which will happen many times!), it cannot disappear in the surrounding vegetation and can easily be secured with a net or a small container.

A good tip to photographing "nervous" specimens is to place an opaque container over them and wait a few minutes. Usually the specimen will stay quiet for some time when you remove the container.

To avoid any confusion between specimens and photographs, we always photograph the tag associated with the specimens before taking photographs of the next specimen. Many digital cameras allow you to assign a peculiar number to each photograph, but we found that method slower and more restricting.

Most recent digital cameras are robust and can be used in the field on condition that it avoids contact with water. Be sure to keep them in waterproof bags or suitcases when you are not using them (see "Carrying food and equipment" above). Always place desiccant in your bags/cases; we use reusable silicagel placed in small transparent containers that have small holes in the lid. This type of silicagel is blue when dry and becomes pink while wet, so you can easily detect changing conditions and when necessary, the time to replace it. The silicagel will become blue again once exposed to high temperature (on the stove for example), allowing for water evaporation.

A digital reflex body camera with a macro lens is a must to photograph amphibians. A wide-angle lens should be used for habitats and microhabitats. The senior author uses a remote macro flash system that gives excellent results (Fig. 25C). We also carry a small shockproof and waterproof compact digital camera that record short video sequences, which may be useful when observing a peculiar behaviour.

Taking good photographs requires some skills and is time-consuming, but efforts are worthwhile!

➔ Basic equipment needed for taking photographs of your specimens:
<ul> <li>Camera body (preferably digital).</li> <li>Macro lens.</li> <li>Wide-angle lens (a 18-70 mm zoom is ideal).</li> <li>Flashes, remote macro flash system is ideal.</li> <li>Several memory cards (or film rolls if you run a non-digital camera).</li> <li>Memory card reader.</li> <li>Batteries.</li> <li>Battery charger.</li> <li>Compact digital camera allowing recording video sequences (optional).</li> <li>Small tent with large front opening.</li> <li>Net to secure the animal if it tries to escape.</li> <li>Laptop and external hard drive or other media storage device to backup your photos and empty your memory cards (optional if you have plenty memory cards).</li> </ul>



**Fig. 25.** Taking photographs of specimens. A. Small tent with large opening used as a "field studio"; B. Digital images are downloaded on a laptop right in the field and saved on an external hard drive; C. Digital reflex with macro lens and remote macro flash system as used in the field by the senior author, note the protective *Peli*<sup>TM</sup> case. (Photos by P. J. R. Kok).

## 3.3.5. Recording of advertisement calls

Male anuran advertisement calls are species-specific, and bioacoustics analyses of frog vocalizations are invaluable in the discovery of new taxa, assessment of taxonomic rank, and species identification (see "Call analysis" below). Frog recordings can even detect species otherwise thought to have been absent in a specific area. In some studies tape recordings may be used as voucher material.

It can be surprisingly challenging to locate a calling frog or toad. Some species call from beneath leaves or under the ground, and in many cases their calls are so ventriloquial that the position of the calling male is very difficult to estimate.

You should always collect the specimen recorded as a voucher and take associated data as recommended in "Field notes and labels" above; do not forget to include tape identification (name of the person making the recording + tape number) and temperature during recording (see below). One voucher specimen per species per calling site is a minimum.

To acquire quality recordings that will allow you to perform reliable analyses you will need a good recording system that offers very ultra-low distortion levels and is immune to speed errors, tape noise, and non-linear frequency anomalies. Dominant frequency of the advertisement call must be accurately captured and a recorder with a flat frequency response from *ca.* 20-15000 Hz is appropriate. A recording level-meter is mandatory to avoid distorted signals due to too high-level recording. Avoid devices that utilize an audio compression algorithm (like Digital MiniDisc recorders). We recommend DAT recorder, Hi-MD recorder, digital hard drive or solid-state recorders. Using an expensive recorder with a low quality microphone is not recommended. Choose a directional, omnidirectional or a shotgun microphone with no noticeable distortion in the 20-10000 Hz range. Preferably use 30 minutes tapes. Headphones will allow you to evaluate the quality of your recordings in the field and should offer some degree of isolation from ambient noises. We use a *Sony* DAT *TCD-D100* recorder with a *Sony ECM-MS907* microphone with very good results.

Note that temperature variation affects call parameters and a critical step in recording frogs is obtaining temperatures during recordings. If a frog is calling in water, water temperature should be recorded. Do not forget to always carry a waterproof thermometer along with your recording equipment.

Single calling individuals or choruses may be recorded. For the purpose of taxonomic research, recording of calling individuals is required. Frogs should be recorded at distances from 0.5 to 1.5 meters using an appropriate gain level (test gain level before recording). Record at least 5-10 calls from each individual and do not forget to keep recording between calls, this will allow you to know the intercall interval (see "Call analysis" below). Before each recording you should add a voice label giving basic information like the name of the person recording, locality, time, temperature and field identification. Some species are very shy and stop calling if they are only slightly disturbed (by your voice for example), you might thus prefer to report these data in your notebook referring only to the number of the recording. Saying "stop" at the end of each recording will help you to locate different recordings from different individuals. DAT recorders allow to

automatically stamp the current date and time on the tape and allow quick access to the starting points of your recordings thanks to an indexing system. The caveat of digital audio recorders is that they are sensitive to high humidity level and might stop working in very humid environments. We always protect the device in a ziplock bag during recordings and place the recorder in a protective case with desiccant (see above) when not in use.

Some species call only during heavy rain, which creates a lot of background noise. Rain falling on your microphone or on the ground next to the frog may be a problem. We use a small umbrella to avoid that trouble.

## → Basic equipment needed for recording frog advertisement calls:

- Recorder.
- Microphone.
- Headphones.
- Batteries.
- Tapes.
- Ziplock bag to protect the recorder from rain.
- Waterproof thermometer.
- Small light umbrella.

## 3.3.6. Euthanasia of voucher specimens

Once voucher specimens have been photographed, they must quickly be killed using a humane method of euthanasia. This for evident ethical reasons and practical motivations: specimens humanely killed will be relaxed and much easier to fix in the proper position. Do not expose your specimens to inappropriate handling, temperature extremes or any other undue suffering. Never place living specimens in formalin without prior euthanasia, their agony will be long and painful and specimens could be contracted making further examination problematical.

Many investigators use a chlorobutanol solution in which the animal is immersed. We prefer to use local anaesthetics like lidocaine or similar drugs that have the advantage of not being controlled substances. Also they usually are easily available in pharmacies in most countries, and are available in a wide range of presentations (injection, spray, gel).

Specimens are immersed for a few minutes in the solution, which must be regularly replaced. Note that amphibians are species-specific in their response to anaesthetic chemicals and that some large specimens (*e.g.* large *Rhinella* or *Leptodactylus* species) may require intracardiac or intraperitoneal injection of the solution.

## → Basic equipment needed for voucher specimens euthanasia:

- Syringes and needles.
- Containers for euthanasia.
- Lidocaine or similar drug.

## 3.3.7. Preservation of voucher specimens

As stated above, good preservation of the voucher specimens will simplify their identification and the description of possible new species; it will also guarantee long-term preservation. Preserving specimens is basically a two-step process: (1) the specimen is fixed in preservative; (2) the specimen is transferred to 70% ethanol for permanent storage. For step 1 we typically use 10% formalin. Pure formalin can be bought in pharmacies or drugstores in many countries and you just will need to dilute it: one part of 100% formalin in nine parts of water will give you a 10% formalin solution. Be careful with formalin because it is irritating, carcinogenic, and very harmful to the environment. Always wear gloves and be careful not to receive projection of the solution in the eyes. This can happen when you inject specimens or simply if a bottle falls on the ground. Formalin in the eyes is a very unpleasant experience and eyes must be immediately washed with water for several minutes. Never abandon formalin in the field.

Ideally the 10% formalin solution should be buffered with magnesium carbonate to avoid acidification or alkalinisation of your fixation solution (use 1/2 teaspoon of magnesium carbonate per litre of 10% formalin). Acidification or alkalinisation will cause excessive discolouration, clearing and/or decalcification of your specimens.

70% ethanol is an alternative fixing solution if formalin is not available.

Step 2 will usually only happen once you are back from the field (see "Collection management" below).

Once the formalin solution is ready you can prepare your fixative trays. We typically use lidded plastic containers of *ca.* 40x25x9 cm (*Really Useful Boxes*®). The bottom of the tray is covered with white tissue saturated with 10% formalin (we use strong cellulose paper or cheesecloth; avoid coloured tissues that could discolour your specimens, see Fig. 26).

Once you are sure that the specimens are killed and completely relaxed – for frog specimens a stimulus on the frog's eye is a good indicator: if the eye retracts, the frog is still living – you must dispose them in a way that will facilitate measurements and further examination of important morphological characters (webbing for instance, see Figs 26, 27A-B). In case of large specimens, you will need to gently inject them with 10% formalin to be sure that they will not partly rotten. Figure 28 shows multiple injection points, and figure 27C positioning of amphibians for final fixation. We usually attach a tag before fixing the specimen to avoid tags and specimens mixing. However, this is not always feasible, especially in small specimens that will not fix in the right position with the tag. In this case, the tag is deposited on the back of the specimen and will be attached

immediately after fixation. Once your specimens are correctly positioned, cover them with another piece of saturated tissue, gently add a little more of fixing solution and cover the tray.



Fig. 26. Fixative tray. (Photo by P. J. R. Kok).



**Fig. 27.** Fixation of specimens. A. Hand of a properly fixed frog, note that webbing is easily examined and that measurements will be taken without difficulty (length of Finger III for example); B. Hand of an incorrectly fixed frog, note that measurements could be approximate and examination of webbing difficult; C. Ideal position of a frog in the fixative solution, which will facilitate measurements and further examination. (Photos by P. J. R. Kok).



Fig. 28. Formalin multiple injection points (red dots). A. In frog; B. In caecilians (here shown on a snake, in which the same method is applied). (Photos by P. J. R. Kok).

After a few hours or a full day, depending on the size of the animal, specimens are hard enough to be transferred to a container filled with 10% formalin (Fig. 29). Check specimens often to judge when the transfer may occur, but do not be afraid to leave them too long in the trays. Specimens will remain in 10% formalinfilled containers until the end of the field trip. We use different sizes of widemouth jars and try to keep together specimens having approximately the same size. Avoid mixing tiny specimens with large ones and be sure to not overcrowd your jars, but do not leave too much space because if you transport the specimens - which will be the case if you move from one location to another with all your equipment - they might be damaged by friction with others. To avoid that, we usually fill the container with soaked tissue, or wrap most fragile specimens with cotton tulle. Fragile specimens can also be kept in separate small vials. Jars and containers must absolutely be kept out of direct sunlight because this will accelerate discolouration, could interfere on the fixation process of the specimens, and could modify the pH of your solution (which will affect your specimens, see above).



**Fig. 29.** When they are hard enough, specimens are transferred to a container filled with 10% formalin in which they will remain until the end of the field trip. (Photo by P. J. R. Kok).

If you are not a local resident, at the very end of the field trip you will probably need to have your specimens checked by local colleagues before exporting them. This is the perfect occasion to pack them for transport. Good packing of specimens is almost as important as fixation because if you are careless you might have disagreeable surprises (specimens desiccated, distorted, etc.).

The best procedure to pack specimens is the following: use large pieces of formalin-saturated cotton tulle to wrap 1-10 specimens together (again do not mix small specimens with large ones). Once the specimens are wrapped, be sure that the packet is wet enough and transfer it in a leakproof plastic bag (Fig. 30). Close the plastic bag tightly. We usually pack specimens by species and by size to facilitate our work in the laboratory. Avoid overcrowding your plastic bags and be careful that toes and fingers of specimens will not be stressed. We usually slightly inflate the plastic bags for shock protection. Insert the bag in a second plastic bag for security, put all the plastic bags in solid waterproof jars - the same you used in the field for your fixative solution - and add a notice for customs with the following text: "This package contains dead, preserved animals for scientific studies that have no commercial value. If this shipment is inspected, it is absolutely imperative that animals wrapped in wet tissue be returned to and sealed inside the plastic bag. If not, the material will dry rapidly and become useless. We thank you very much for taking good care of this invaluable resource".

Specimens are now ready to be shipped to the laboratory.



Fig. 30. Packing of specimens for transport. A-C. Specimens are wrapped in formalinsaturated cotton tulle; D. The packet is well soaked and transferred in a leakproof plastic bag. (Photos by P. J. R. Kok).

→ Basic equipment needed for preserving and packing voucher specimens:

- Full-strength formalin (ideal), 70% ethanol (alternative).
- Buffer for formalin (magnesium carbonate).
- Plastic teaspoon.
- Forceps (long and small).
- Dissecting scissors.
- Syringes and needles (various sizes).
- Preserving trays with lids.
- Tissue (strong cellulose paper or cheesecloth).
- Cotton tulle.
- Leakproof plastic bags.
- Nitril gloves.
- Wide-mouth air/watertight bottles for fixation solution.
- Wide-mouth air/watertight jars for fixation solution, storage of large specimens and shipping.