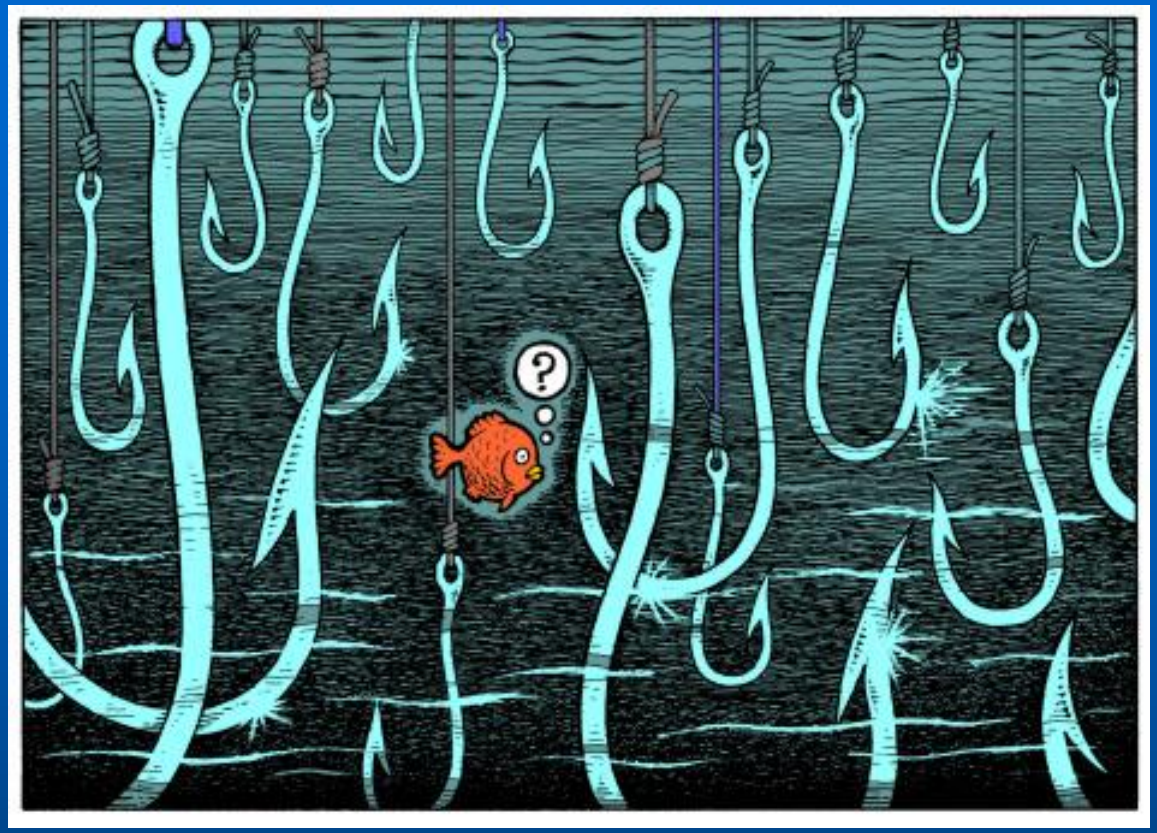


Fish Taxonomy

- Making a new fish collection
- Collection management

Emmanuel Vreven

Royal Museum for Central Africa (RMCA Tervuren)

FishBase and Fish Taxonomy Training Session 2018

“If field workers understand how specimens are processed and used in museums, they will prepare better specimen. If collection users understand how animals are collected and preserved in the field, they will make better use of the specimens. If all of us understand how collections are managed, specimens will be better utilised and preserved for the future.” (Simmons, 2002)

- Making a new collection
Preparations: What will you need?

Paperwork (permits & licences):

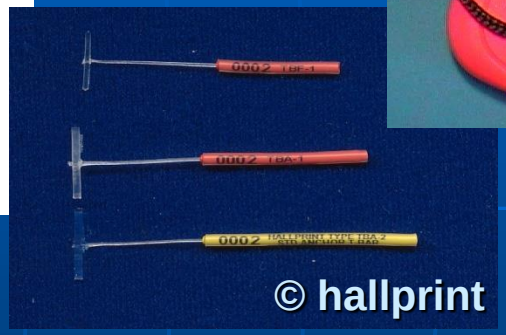
- (i) **Cites** (Convention on International Trade in Endangered Species : only a single species: *Caecobarbus geertsii*.
- (ii) "**Ordre de mission**": restricted in geographic cover + participants.
- (iii) Additional permits needed to collect in **National Parks**.
- (iv) Additional permits are also needed for (1) electrofishing and (2) rotenone (ichthyotoxine) sampling.
- (v) **Health or veterinary certificates** (for exporting as well as importing country): explicitly mention: "specimens and samples".

Note: print all required documents in all official languages of the country.

- Making a new collection
Preparations: What will you need?

- Gill-nets, traps, electrofisher, rotenone, fykes, casts nets...
- Formalin (10 %) (powder) (careful handling (noxious & carcinogen): use nitrile or neoprene gloves for protection)
- Barrels (± 25 L)
- Plastic bags & containers + elastics (both of different sizes)
- Formalin resistant paper and pencil
- Eppendorfs with alcohol (95%) (for fin clips: DNA analysis)
- Tags and Applicators (pistol) (expensive, 1000 tags: ± 400 Euro for => alternative, Dymo label printers: ± 50 Euro)
- Camera + aquarium + clove oil or MS 222 (anaesthetic)
- Detailed maps
- GPS (± 120 Euro)

- Making a new collection
Preparations: What will you need?



- Log-books [(1) locality: village, road, river basin, river, affluent etc…; (2) DNA sample and (3) photographs log-book)]
- Dissection kit (scalpel, scissors, needles etc...)
- Necessary documents (local legislation): « Ordre de Mission »

Collecting methods: Gillnets

- A battery of different mesh sizes: 8, 10, 12, 15, 20, 25, 30, 35, 40, 45 and 50 mm (30 m length and 1.5 m depth)
- Night (morning) and day (afternoon)
- Do not try to large rivers (the nets will be hit by branches or wood or will simply not resists to the pressure of the water especially not if leafs or algae are trapped in the nets)

- - damaging the fish
- - the fish dies in the net (quality of the collected specimens photographs of dead specimens)

- + sampling in very large rivers
- + capture of large fish specimens

Collecting methods: Electrofishing

- Electrofishing = the use of electricity to capture fish.

- Electricity: be careful!

- - depending on the conductivity of the waters to be sampled
- - time consuming
- - heavy work (weight of the electrofisher)
- - expensive (± 5500 Euro)
- - need of electricity (charge on the engine of the 4x4)
- - "invisible" harm to fishes

- + « non » selective
- + well preserved specimen
- + fresh specimens

Collecting methods: Electrofishing

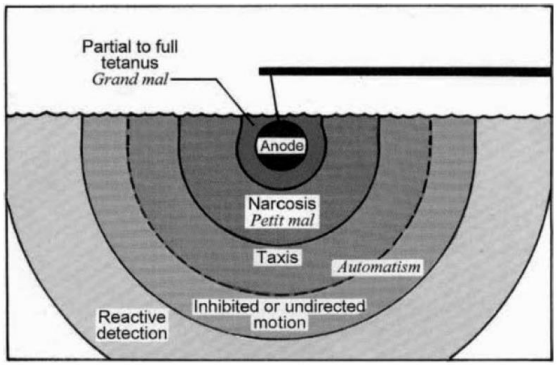


Figure 1. Major intensity-dependent electrofishing response zones. The outer boundaries of response zones for a spherical anode at the surface and sufficiently distant from the cathode are more-or-less hemispherical shells around the anode that represent field-intensity thresholds for the associated responses. Actual and relative sizes of the zones are specimen dependent (species, size, condition, and orientation) and vary with electrical output, electrode size and shape, and environmental conditions. Labels in italics represent corresponding phases of epilepsy as suggested by Sharber and Black (1999) except that here the phase of tonic–clonic contractions (quivering or pseudo-forced swimming) between petit mal and grand mal (narcosis and tetany) is treated as the initial part of grand mal (partial tetany). Zone of reactive detection is sometimes referred to as zone of perception. Zones of taxis, narcosis, and tetany represent the effective range for fish capture using direct and pulsed direct currents. (Reproduced from Snyder (2003), Figure 11.)

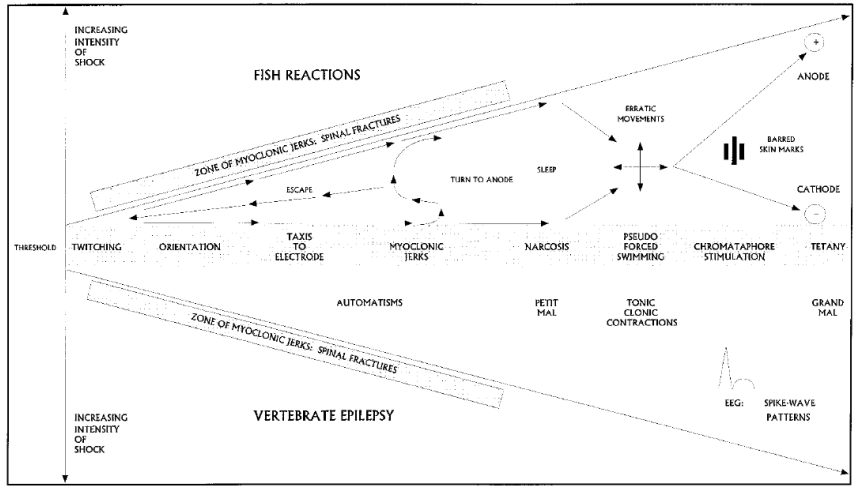
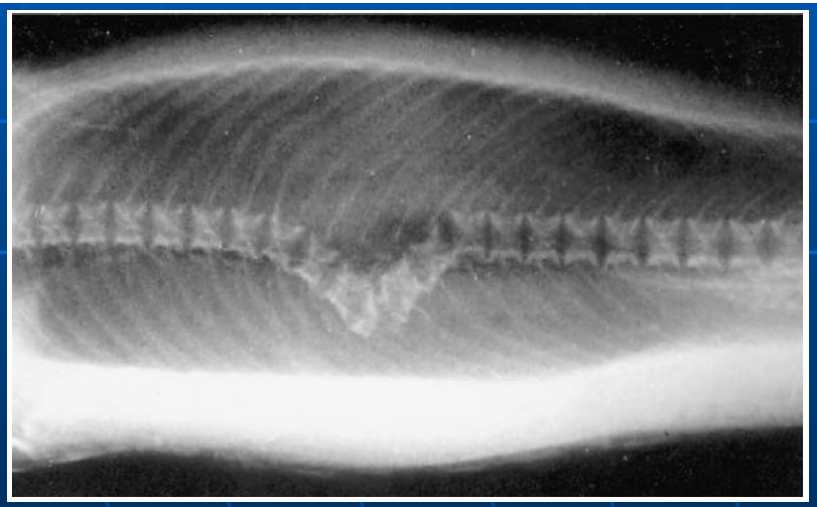
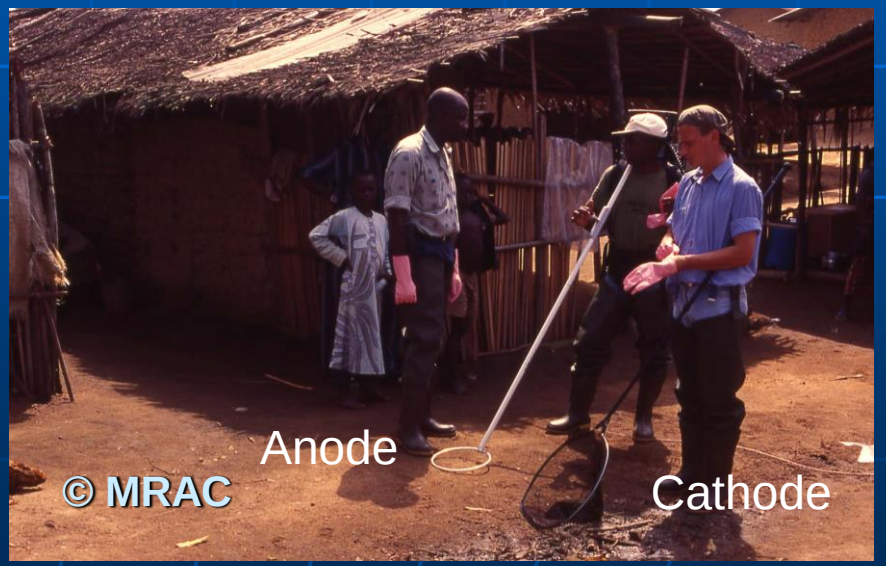


FIGURE 1.— Diagrammatic comparison of rainbow trout reactions, electrofishing terms, and vertebrate epilepsy terminology.



Snyder, 2004
Sharber & Sharber Black, 1999



Collecting mehods: Rotenone

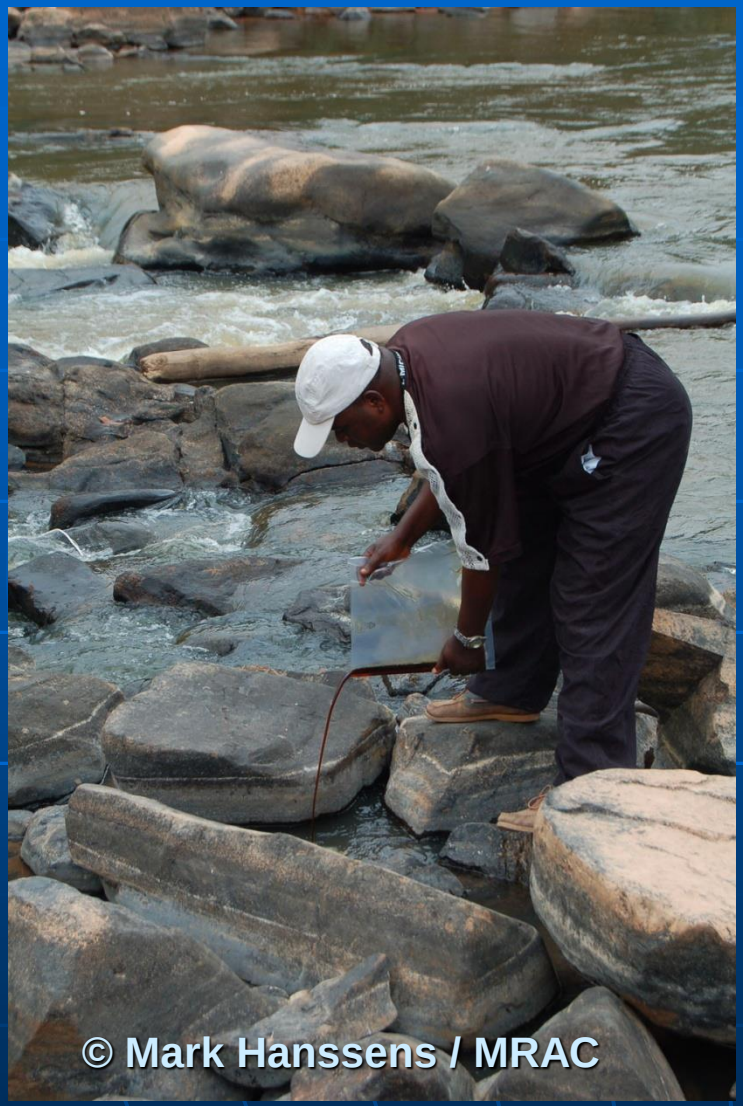
- Ichthyotoxine
- (alternative: use of local ichthyotoxines/ichtyocide)

- - select an area of the river (± 25-50-100 m)
  - Water not to deep (± 25-50 cm)
  - Preferably water with good visibility (floating, but also sinking of affected specimens)

- - gill nets (small mesh)
  - One above selected area of the river
  - At least two below selected area of the river (± 50 - 100 m)

- - be careful: add low doses (effects downstream!) - add enough (fish will try to escape)

Collecting methods: Rotenone



- Powder form
- Liquid (emulsified liquid)

Collecting methods: Rotenone

Collecting methods: Rotenone

- - very expensive (about 750 Euro for 30 litres)
- - general negative perception by local community (fishermen often positive perception)
- - not possible to sample in all habitats (area free of thicker mud deposits: specimens easily covered by disturbed mud)

- + « not » selective (note: all methods selective : different sensitivity of the species to the product)
- + fast (time consuming / help of local fishermen or children)
- + fish is alive (life aquarium photographs)
- + fish not damaged by gillnets (good quality specimens)
- + possibility to collect in « radiers »

Collecting methods: Local fish gear

- **Fish Biodiversity:**
  diversification of methods and habitats
- **- cast nets**
- **- traps**
- **- angling (children)**

Collecting methods: local fish market

Landing, sorting & euthanizing of the catch

- Catch must be landed carefully to avoid damage
- Catch must be removed form entanglement as carefully as possible also to avoid damage: i.e. (i) descaling; and (ii) broken spines and/or serration
- Be aware that some species have poison glands
- Rapid sorting of the catch is crucial:

- (i) to obtain an overview if species and specimen numbers per sex, size etc… ;

- (ii) to separate specimens for DNA sampling, photographing from the remaining ones;

- (iii) to release or fix immediately the remaining specimens (especially small sized specimens quickly deteriorate at high temperature)

- Euthanize with clove oil or MS 222: only those specimens that can be processes immediately (deterioration + post mortem colour pattern changes)

Log-books

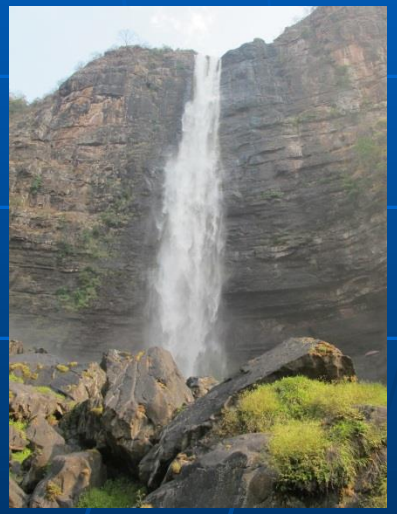
MRAC 2017-015

Localité	date	coordonnées photos! EV	t°	pH	TSD	Cond	Sal	Nbre de spécimen
Grotte de Kiamvu / Village de Kiasi Mankala	15/08/2017	Lat: 05°21'37,8"S; long 14°55'40,1"E; Alt. 630 m	21.1	10.48	343.6 ppm	700.2 µS/cm	0.390 psu	5
Grotte de Vungu / Village de Ntandalanga	15/08/2017	Lat: 05°19'2,2'' S; Long: 14°51'55,0'' E; Alt. 550 m	23.8	8.44	257.0 ppm	525.1 µS/cm	0.304 psu	3
Grotte de Muisi / Village de Kitalampanga	16/08/2017	Lat: 05°49'44,3" S; long: 14°55'57,3"E; Alt. 641 m	22.4	9.22	143.4 ppm	291.7 µS/cm	0.190 psu	3
Grotte de Mambuela / Village de Kongo dia Kati	17/08/2017	Lat: 05°44'32,5" S; Long: 14°54'17,2" E; Alt. 618 m	20.0	8.93	296.0 ppm	597.6 µS/cm	0.327 psu	3 + 1 (2 Alexis)
Grotte de Nkotua Nkiende / Village de Nsangi	17/08/2017	Lat: 05°44'20,5" S; Long: 14°52'57,1" E; Alt. 533 m	21.6	9.36	286.5 ppm	612.8 µS/cm	0.312 psu	5

Collection 2017-015

	position	tube n°	species	date	loc.		Expédition	Collection	tag
1	A1	AB6256373 1	Caecobarbus	15/08/2017	Village Ntandalanga, Grotte de Vungu (Zone I)	Zone I	Cave Expédition 2017	2017-015-P	= tube n°
2	B1	AB6256373 2	Caecobarbus	15/08/2017	Village Ntandalanga, Grotte de Vungu (Zone I)	Zone I	Cave Expédition 2017	2017-015-P	= tube n°
3	C1	AB6256373 3	Caecobarbus	15/08/2017	Village Ntandalanga, Grotte de Vungu (Zone I)	Zone I	Cave Expédition 2017	2017-015-P	= tube n°
4	D1	AB6256373 4	Caecobarbus	15/08/2017	Village Kiasi Mamkala, Grotte de Kiamvu (Zone I)	Zone I	Cave Expédition 2017	2017-015-P	= tube n°
5	E1	AB6256373 5	Caecobarbus	15/08/2017	Village Kiasi Mamkala, Grotte de Kiamvu (Zone I)	Zone I	Cave Expédition 2017	2017-015-P	= tube n°
6	F1	AB6256373 6	Caecobarbus	15/08/2017	Village Kiasi Mamkala, Grotte de Kiamvu (Zone I)	Zone I	Cave Expédition 2017	2017-015-P	= tube n°
7	A2	AB6256373 7	Caecobarbus	15/08/2017	Village Kiasi Mamkala, Grotte de Kiamvu (Zone I)	Zone I	Cave Expédition 2017	2017-015-P	= tube n°
8	B2	AB6256373 8	Caecobarbus	15/08/2017	Village Kiasi Mamkala, Grotte de Kiamvu (Zone I)	Zone I	Cave Expédition 2017	2017-015-P	= tube n°
9	C2	AB6256373 9	Caecobarbus	16/08/2017	Village Kitalampanga, Grotte de Muisi (Zone II)	Zone II	Cave Expédition 2017	2017-015-P	= tube n°
10	D2	AB6256374 0	Caecobarbus	16/08/2017	Village Kitalampanga, Grotte de Muisi (Zone II)	Zone II	Cave Expédition 2017	2017-015-P	= tube n°
11	E2	AB6256374 1	Caecobarbus	16/08/2017	Village Kitalampanga, Grotte de Muisi (Zone II)	Zone II	Cave Expédition 2017	2017-015-P	= tube n°
12	F2	AB6256374 2	Caecobarbus	17/08/2017	Village Kongo dia Kati, Grotte de Mambuela (Zone II)	Zone II	Cave Expédition 2017	2017-015-P	= tube n°
13	A3	AB6256374 3	Caecobarbus	17/08/2017	Village Kongo dia Kati, Grotte de Mambuela (Zone II)	Zone II	Cave Expédition 2017	2017-015-P	= tube n°
14	B3	AB6256374 4	Caecobarbus	17/08/2017	Village Kongo dia Kati, Grotte de Mambuela (Zone II)	Zone II	Cave Expédition 2017	2017-015-P	= tube n°

Photographing



Fixation

- (i) Fixation & (ii) preservation are processes used to prevent postmortem changes.
- Formalin (10 %) (fine powder not small pellets). By definition all fixatives damage DNA.
- Large fishes (> ± 20-25 cm) should be injected with the use of a syringe, otherwise the preservative will not penetrate all the tissues before decay sets in. Especially important in the tropics and for some groups [e.g. *Labeo, Labeobarbus* & *Varicorhinus* (Cyprinidae)]. Inject at the base of the pectoral/pelvic fins into the belly (advantage: no scales). Eventually a small slit can be made along the belly.
- Cichlids sometimes open their mouth before dying due to the lack of oxygen in the water. While the fish are fresh you can still close the mouth and fix the mouth with a little needle you can remove once the fish is well fixed (also applicable to other fishes with open mouth). Otherwise the mouth easily opens during fixation causing measuring problems afterwards.
- Formalin should be handled with care as it is a noxious chemical which irritates the eyes and nose and is painful in skin cuts.
- If possible, let the fish float for a few days in the plastic bag/container before stocking them into the barrel. Do not put to much fish in one bag!

Preparation for transport

- Remove almost all liquid (i.e. formalin 10% solution). Just leave enough liquid so the fish do not dry out.
- Plastic barrels should be well closed to avoid leakage during transport.

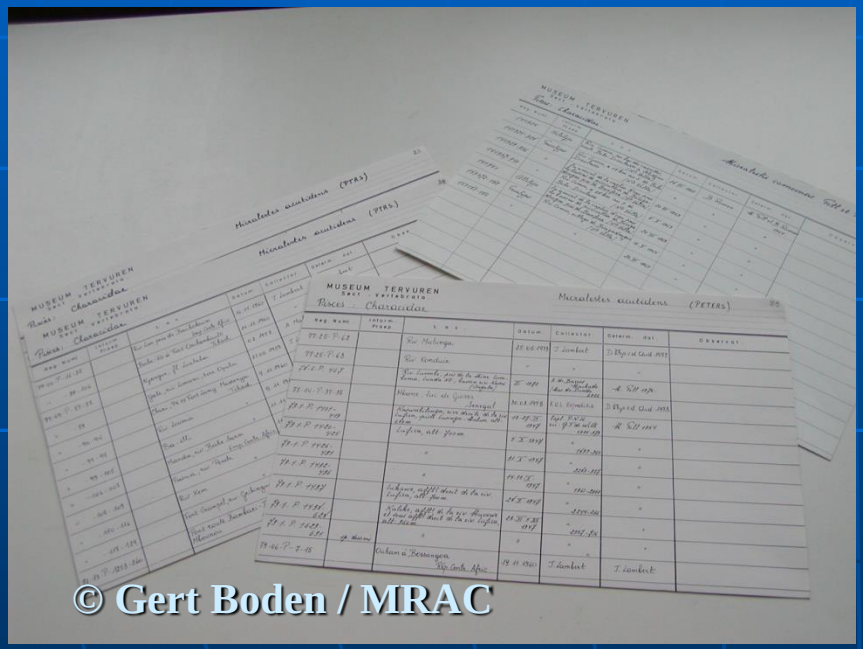
Fixation

- Collection Management

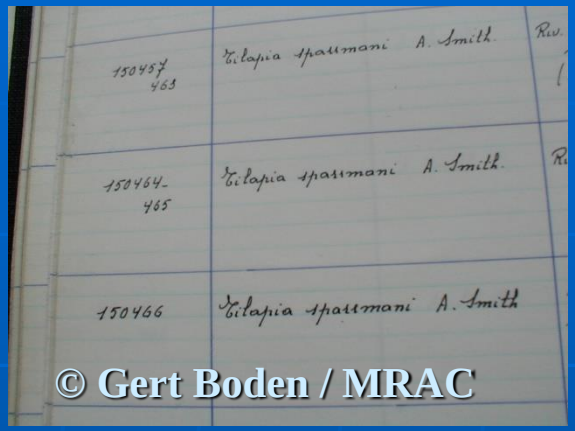
- **Arrival (Spoel lokaal)**

- Rinse the specimens in water during a few days (=> this to remove excess of formalin). Verify that the formalin smell has really gone. Specimens which are transferred to early to alcohol will keep their unpleasant formalin smell for handling afterwards. Formalin is noxious!
- Transfer to 70% alcohol (+ camphor) = Preservation.
- Fluid preservation is a two-step process of fixation (see above: formalin) and preservation (alcohol).
- Sorting of locality samples by family, genus and preliminary “species”. => Copy the locality data to label each of the subsamples made. + Add the unique collection number!

Double system: paper record and computer record

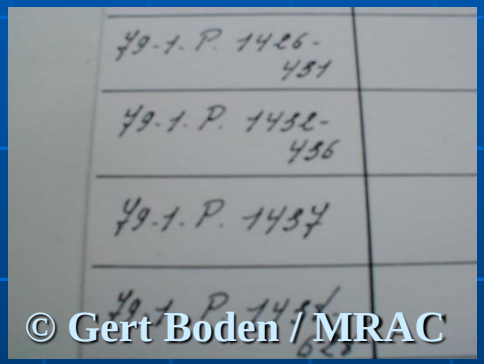


Individual collection number and specimen numbers

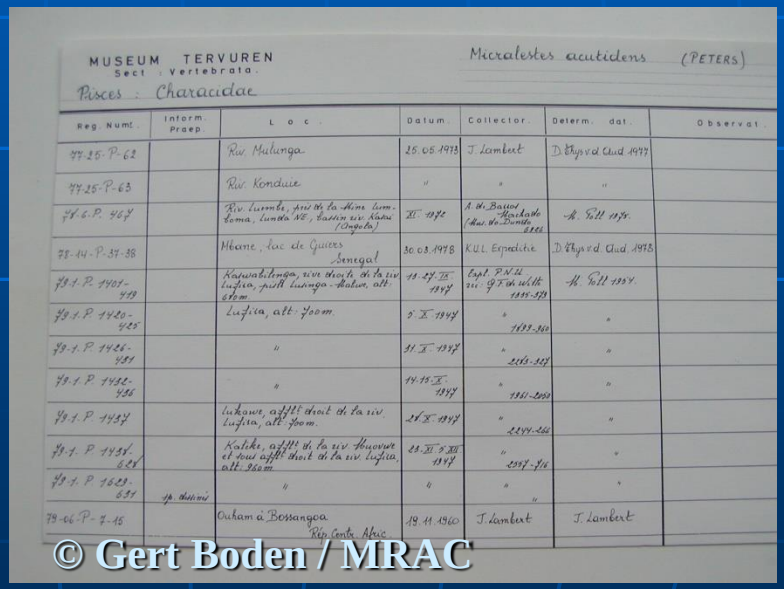


© Gert Boden / MRAC

© Gert Boden / MRAC



© Gert Boden / MRAC



© Gert Boden / MRAC

Year (of entering) (e.g. 79)
Collection number (e.g. 1)
P = Pisces
Specimen number(s) (e.g. 1437)

Labels

- Collection number(s)
- Information on the preparation [alcohol, cleared and stained, skeleton… size of the fish(es)]
- Locality (Country)
- Coordinates: if provided by collector(s) => try to make a difference between provided coordinates by collector(s) or subsequently added coordinates
- Collector(s)
- Date
- Identifier => This implicitly somehow enables to evaluate the quality of the identification
- Remarks: reidentifications, collecting method, DNA sample, microhabitats stations, etc…

- Note: Keep the old labels, i.e. the ones with previous identifications, in the jar. Never remove labels from a jar.

Types: red labels

Collection

- By family (« phylogenetically ») / place for unidentified specimens
- By genus (alphabetically) / place for unidentified specimens (sp.)
- By species (alphabetically)

* Reorganization might be time consuming

Detailed logbook of exact location within the collection

Further reading

- **Coad, B.W. 1995**. Expedition Field Techniques FISHES. Published by the Expedition Advisory Centre Royal Geographical Society. 97p.
- **Neumann, D. 2010.** Preservation of freshwater fishes in the field (Chapter 22). In: J. Eymann, J. Degreef, Ch. Häuser, J.C. Monje, Y. Samyn & D. van den Spiegel (Eds). Manual on field recording techniques and protocols for All Taxa Biodiversity Inventories and Monitoring. ABC Taxa, Vol 8, part 2: 587-631.
- **Simmons, J.E. 2014.** Fluid Preservation. A comprehensive reference. Rowman & Littlefield. 347p,