

Chapter 21

Tips for bird surveys and censuses in countries without existing monitoring schemes

by

Friederike Woog

State Museum of Natural History Stuttgart
Rosenstein 1, 70191 Stuttgart, Germany
Email: woog.smns@naturkundemuseum-bw.de

Swen C. Renner

Institute of Experimental Ecology, University of Ulm
Albert-Einstein Allee 11, 89069 Ulm, Germany
Email: swen.renner@uni-ulm.de

Jon Fjeldså

Zoological Museum, University of Copenhagen
Universitetsparken 15, DK-2100 Copenhagen, Denmark
Email: JFjeldsaa@snm.ku.dk

Abstract

Birds are important environmental indicators and, for a long time, have been included when surveying biodiversity. This has led to a whole array of publications, some of which are available over the Internet, making them easily accessible worldwide. Here we provide practical guidance, with relevant source references, for how to plan and conduct bird surveys and censuses, especially in tropical environments.

Keywords: biodiversity, bird counts, assessments, monitoring techniques

1. Introduction

When surveying biodiversity, birds are usually included because they have been more completely charted (in terms of taxonomy and distributions) than any other taxonomic group, and because good field guides, and even bioacoustics data, are available for identification for most parts of the world. Furthermore, a large number of skilled birdwatchers are often keen to volunteer in bird monitoring projects. In order to develop bird monitoring as an effective tool in conservation biology, a whole array of literature about bird census and monitoring techniques has been published, the standard book by the late Bibby and his colleagues (2000) covering most of them.

In 1998, Bibby *et al.* presented a guide especially designed for expeditions. Gilbert and a team of specialists published a comprehensive book on monitoring techniques for all sorts of UK bird species ranging from songbirds to raptors to waterfowl (Gilbert *et al.*, 1998). A “Best Practice Guide for Wild Bird Monitoring” was published in 2008 by Voříšek and his colleagues focusing mainly on Europe and giving an overview of existing monitoring schemes. It can be downloaded, making it easily accessible to birdwatchers and ornithologists worldwide (<http://www.ebcc.info/index.php?ID=365>). More articles with information on specialized count procedures can be found in Gibbons & Gregory (2006). So why include birds in this manual? The goal of this article is to give practical advice on how to plan bird surveys and censuses in countries where monitoring schemes are lacking, and to provide useful Internet links. This is by no means a complete treatment of methods, study design, data management and analysis as this would be far beyond the scope of this chapter. All of these, sometimes rather complicated topics, are covered well in the publications mentioned above (also see Gregory *et al.*, 2004).

This chapter is written from a European perspective. Whereas the general biology and life cycle of European and North American birds is rather well known, we know far less about birds from many other parts of the world. It is useful and important that scientists from developed countries contribute to the study of biodiversity outside Europe, especially in the tropics. To be sustainable, the long-term monitoring in developing countries should be locally based (Danielsen *et al.*, 2006, 2007). Although hard to achieve, this goal should always be kept in mind, and is indeed often feasible once local communities experience how simple monitoring systems can be used proactively to manage their own resources.

2. Preparation for the survey

To start with, we advise contacting local ornithologists, to tell them about the plans and to ask them if they support the idea of a survey and if they would become a partner. It is important to find the right people to work with, people that are accepted locally but ideally biologists by training. In most countries birdwatchers and ornithologists are associated with the Birdlife International Partner and can be tracked down by visiting the Birdlife International homepage, a network of birdwatchers and ornithologists worldwide :

<http://www.birdlife.org/worldwide/national/index.html>. A local partner can help to answer the following questions: (1) Have surveys already been carried out in the target area or are some being planned? (2) Do other monitoring schemes already exist? (3) When is the best time for a survey (season and time of day)? (4) Are permits needed and how are they to be obtained? If a certain survey or monitoring scheme is already in place one should consider choosing a similar method to make data comparable between sites. A good example for a large-scale bird survey is the second South African Bird Atlas Project SABAP2 (<http://sabap2.adu.org.za/index.php>). On the homepage there are good descriptions of survey methods and databasing procedures including various downloads.

For your partnership to work, consider that volunteer schemes, as developed in Europe and South Africa (breeding bird surveys, international waterfowl counts etc.) rarely exist in developing countries. People have to work to survive and can rarely afford a hobby like birding. Therefore, find out what the “normal” fees and salaries are. Discuss this issue with your local partner beforehand. Out in the villages, it may be useful to make agreements about donations and salaries with a village chairman, or council, rather than with the individual helpers. Such discussions may be cumbersome (for you) and you may feel awkward, but nothing is worse than having to sort out conflicts afterwards. Sometimes it can be useful to have a small contract telling the nature of your cooperation who is responsible for which aspect, signed by all parties involved and a copy resting with each party.

2.1. Species identification

When planning a survey in an unfamiliar region, prepare yourself beforehand, as this will save a lot of time in the field. This includes surveying existing ornithological literature about birds in the target area, and to identify species of particular interest. For most parts of the world there are field guides for birds and CDs with bird calls and songs. The quality of these guides greatly varies and they rarely include juvenile birds. Some are heavy to take into the field. A simple although somewhat drastic trick is to ask a book binder to split your book into two – one with the plates (to bring into the field) and one with the more extensive text that you may decide to leave at home or at base camp. Some publishers have already caught up with this idea, *i.e.* for West Africa (Borrow & Demey, 2004), New Zealand (Robertson & Heather, 2004) or South Asia (Rasmussen & Anderton, 2005). A good source to check what species occur in an area is <http://www.birdtours.co.uk/> which is a collection of trip reports by travelling birders all around the world, including up to date maps, tips on where to stay and who the useful local contacts are.

2.2. Calls and Songs

We very much recommend using a MP3 player with headphones and microphone that can easily be taken into the field. With a special amplifier, a directional microphone can be used, increasing the range and quality of the recordings. The calls from a CD can be transferred onto the player. Most

modern recordings will offer files in mpg-format. If not, the sound files on audio CDs can be transcribed into mpg-files easily, using freeware available (*i.e.* <http://www.freerip.com/>). Do observe copyright laws and make sure your download is really for free. Free resources are online sound libraries:

http://www.xeno-canto.org/africa/index_static.html

http://www.xenocanto.org/index_static.html

http://www.xeno-canto.org/asia/index_static.html

http://www.xeno6canto.org/australasia/index_static.html

If you are after species from an area where sound recordings are not yet available, you can check with the Wildlife Section of the British Library National Sound Archive (NSA, <http://www.bl.uk/soundarchive>) or the Library of Natural Sounds (LNS) at Cornell Laboratory of Ornithology (www.birds.cornell.edu). Fees may apply. Once you have all songs and calls you need, arranging the files in folders is useful, so that they can be easily found when in the field (*i.e.* in alphabetical or systematic order, whatever the preference). Time permitting, calls of species one expects in the target area can be put into a separate folder. The recording function of most players is usually good enough to make a (low quality) recording of a bird call or song that cannot be identified at once. These can later be sent to a specialist to aid identification. Take a player with regular AA or AAA batteries as those usually can be bought in most countries or, when sunny enough, use small solar battery rechargers. Avoid complicated recharging systems that you need electrical power and adaptors for, as electrical power may not be available at base camp. In the headphone slot one can usually plug small active speakers (working with batteries) that can be used for playback. We do not recommend the use of playback but for certain species it may be necessary, especially to detect cryptic or understorey species. Playback can disturb birds, especially in the breeding grounds. Therefore it should be only used if absolutely necessary and then only very briefly, *i.e.* for a maximum of five minutes. As soon as there is a reaction, stop. Keep in mind that a bird may not visibly respond, yet may still be disturbed.

2.3. Bird collections in museums

Bird collections are good places to brush up bird identification skills before going into the field. If a survey is for scientific and conservation purposes, most museums will allow such studies. See for example:

<http://www.museum.lsu.edu/~Remsen/AVECOLlections.html>

http://www.scricciolo.com/European_Bird_Collections_C%20S%20Roselaar.pdf

To find out whether a museum holds the required specimens, the bird curator should be contacted well in advance and arrangements made to see specimens. Bird curators or collection staff should explain the best way how to handle bird skins. Always handle them with great care because they are meant to be used by generations to come! A large number of web pages are useful to study birds. Many of them are accessible through the GBIF platform (<http://www.gbif.org/>). A useful searchable database is <http://avibase.bsc-eoc.org> giving links to selected Google images, distribution maps, taxonomy, ITIS, Birdlife and Wikipedia. ORNIS, the Ornithological Information System, is linked to GBIF and allows searching 42 mostly American bird databases, including museum specimens (<http://olla.berkeley.edu/ornisnet/>). The site offers

a list of the respective curators including email addresses. For the Neotropics, another good source is <http://neotropical.birds.cornell.edu/portal/home>. The Zoological Museum, University of Copenhagen offers online access to birds collected in Tanzania (http://www.zmuc.dk/VerWeb/Tanzanian_Vertebrates/TanzVert.index.html).

2.4. When to count

The timing of a bird survey will depend on the life cycle of a particular bird species if monitoring one species. Of course, many surveys would be for more than one species. To get an inventory for a given area, typically the major breeding season is best suitable for monitoring the community. Some birds migrate and will be absent from the chosen study area for part of the year. This can include a smaller scale *i.e.* altitudinal movement, but can range to long-distance migration. For most surveys, the time when males are singing on their territory and the birds constructing their nests, are the most suitable, since birds are most active then. Once sitting on the nest, birds often become very silent and cryptic, making it hard for anybody to detect them. In many tropical areas breeding seasons are not as synchronized as in more temperate regions. This means that often only some bird species are breeding whereas other, often closely related species may breed much later. Furthermore, only some individuals within a population may be breeding. The timing of breeding greatly depends on the altitude of your study site and the weather. Even rainforests can be rather dry in certain years. Humidity greatly affects food availability and triggers the onset of the breeding season. Low temperatures in mountain regions can defer the onset of the breeding season. It is often very hard to predict these patterns, even for experienced local people. If logistically possible, we recommend to count at least two times a year.

2.5. Target species

You should aim to find all species possible including breeding birds, wintering birds, cryptic species, nocturnal species, understory species, rare species, bird colonies and mixed species flocks. Special methods are available for most or can be adapted from closely related species (see Gilbert *et al.*, 1998). The more time you spend in an area, the more species you are likely to find. These species discovery curves (Fig. 1) are quite useful as they help you to identify the point in time when the number of new species discovered in an area becomes rather low. For economic reasons and depending on the question being investigated, one may decide to stop data collection at that point (see below for further details).

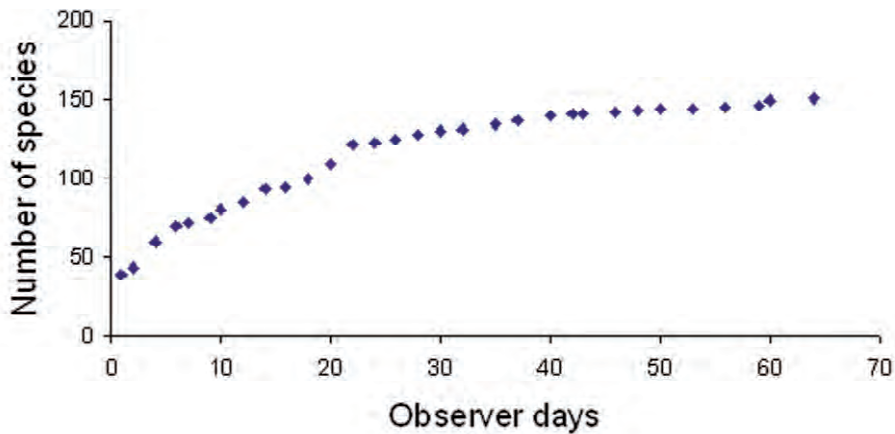


Fig. 1. Species discovery curve, with the total cumulative number of species discovered each day (the time unit could also be hours). At first, the number of species increases rapidly until, after a certain number of days, this number levels off. After that it takes many days to discover a few more species, some of which may only be occasional visitors of the area (after Bibby *et al.*, 1998).

2.6. Habitat

Birds live in almost any habitat you can think of. Clearly, you need to adapt your survey method to the habitat. As an example of two extremes, consider a desert with very low vegetation on one hand, and a dense rainforest with trees ranging up to 40 m into the sky on the other. It is therefore useful to split your survey area into different habitats (*i.e.* forest, scrub, desert, alpine etc.) and to adjust your survey method accordingly (*i.e.* spacing of transects, distance of sample points etc.). If you want to compare different habitats you should, however, use the same protocol. Bibby *et al.* (1998, 2000), Gilbert *et al.* (1998), Gregory *et al.* (2004) and Gibbons & Gregory (2006) offer good advice on this topic.

2.7. Maps

High-resolution maps are essential for a good survey and we recommend obtaining maps prior to surveys. In the capitals of most countries you can contact ministries for geography/geology or local cartographic services. Google Earth images can give you a good idea of your study area, and for many areas high-resolution images are now available. Sometimes they are a few years old and therefore of limited value in areas under rapid “development”.

2.8. Bird behaviour

Birds have very different life styles. Some spend almost all their time up in the air (*e.g.* swifts), some are flightless (*e.g.* Kakapo, Kiwi) or virtually so and skulk around in the understorey vegetation. Birds that live up in the canopy are often almost impossible to detect, as are birds that hide in dense foliage. A few birds

are rather curious and easy to detect, while others are extremely shy. Species detectability depends very much on bird behaviour but also on weather conditions and the skill of the observer. Observers who recognise all of the bird calls and songs of an area will naturally discover more bird species than observers without these skills.

Birds also sing at very different times of day, some start very early in the morning, or advertise their territory around daybreak by giving a single call, some sing at night (e.g. owls). Whereas for some species it may be useful to count breeding territories identified by their territorial song, for other species it may work better to count them during foraging or when they are flying to a roost (i.e. gulls or terns). Many tropical forest birds move around in multi-species feeding parties, or become active only when such parties pass through their territory and provide effective antipredator vigilance. Thus, it is of great advantage to pay attention to these bird parties, which often follow the same route day after day (Poulsen, 1996). In the neotropics, mixed flocks are known to follow ant swarms (Vallely, 2001; Roberts *et al.*, 2000).

Although the books by Bibby *et al.* (1998, 2000) and Gilbert *et al.* (1998) offer more detailed guidance, the annual cycles of birds outside Europe and North America are, in comparison, poorly known. Indigenous people are often the only ones that can give you some ideas about certain species (Ng'weno, 2008). Sometimes their stories may make Europeans sneer because they contain a lot of mystery. Don't sneer at the stories but try to interpret them. A bird spending winter in a tree cavity and coming out of its hole when the thunder arrives could simply mean that it is a migratory species returning with the rains. Documenting all bird behaviour and observations during surveys is a very worthwhile exercise!

2.9. Local knowledge and training of locals

Once in the field, it is very useful to ask a local guide, hunter or project partner to accompany you and to talk to local communities. Going through a bird book with indigenous people can give you priceless information. Depending on where in the world you are, birds are often part of the day-to-day diet of people, so they may have extensive knowledge about them. Talking to the elders of a village one may also find out which species used to occur in an area but may now have become scarce or have disappeared (Ng'weno, 2008). However, when it comes to smaller, similar-looking species, locals often cannot distinguish them as they lack binoculars. It is useful to bring extra binoculars that you can give to local guides or other project participants. To get hold of them ask friends and colleagues back home if they have a pair they don't need anymore. The most important achievement of your trip may not be your species list, but instead the training of locals in bird identification, to make them interested and to possibly teach them how to carry out a monitoring scheme themselves (<http://monitoringmatters.org>, <http://www.springerlink.com/content/100125/>) (Danielsen *et al.* 2005, 2006). Like other monitoring approaches, locally-based methods may be less precise and biased, but may on the other hand be very effective tools for locally based resource management, once locals realize how data can be used for empowerment, e.g. for rapid management decisions to counter habitat loss by

interventions from foreigners and from corrupt administrations. Typically, data collected by locals may lead to prompt and local decisions, while data collected by scientists feed into long-term government regulation (Danielsen *et al.*, 2007). Thus, a combination of both is needed.

2.10. Personal safety

In remote areas always take somebody with you. Local people often know an area very well and have a fabulous sense of orientation. Put an emergency mechanism in place in case you do not return in time. Let other people know where you are going and how long for. When discovering a rare animal the temptation to leave known ground becomes very high and suddenly you don't know how to get back. If one person remains on a path, the second person can go off in search for the animal, remaining in shouting distance you will always find your way back. A handheld GPS and a compass are very useful, but you need to know how to use them. Familiarize yourself with these on known ground. It is good to note the direction of larger roads, or rivers, mountain ranges, steep valleys as they can lead you back in case you get lost. Always think about the basics: enough water, emergency food, sun and mosquito protection, small headlamp (LED's), raingear if needed, small first aid kit, waterproof matches etc. A very powerful yet lightweight torch is the Supernova run with LED's which can function as a signal light (or to be used as a spotlight to see owls...).

Before your trip make sure you have all necessary vaccinations. When mist-netting this includes one against rabies, as bats that are sometimes caught are known to have transmitted this disease. Make sure you have enough medication for all likely diseases with you. When leaving medication behind give it to a local doctor or hospital. Being in remote places it is always useful to know first aid and to be able to diagnose diseases, not only concerning yourself but also the people that work with you (Werner, 1979; Merry, 1994; AAOS, 2007). If one does become sick, one should always go to a doctor. Usually, local doctors have a very good knowledge of local diseases.

3. Short overview of methods available

For anybody planning a survey we strongly recommend to thoroughly study Bibby *et al.* (1998) as it offers in depth advice on many relevant topics (<http://biology.kenyon.edu/courses/biol229/fieldmanual%20birds.pdf>). The best way to learn is to join a professional team for a few days, to get some first field experience and training. The Tropical Biology Organisation offers a wide range of training courses (<http://www.tropicalbiology.org/>).

There are methods that will give you an idea of the species present in an area (qualitative data) but not how many of them (quantitative data). When introducing the factor "time" or "space" into a simple species survey, you can very quickly improve the quality of your data (species discovery curve, encounter rates, MacKinnon index or timed species counts, see Table 1). Quantitative methods are, in general, more time consuming and require more

skill. There are point counts, line counts and even the mapping of territories. In quantitative methods often the distance between observer and bird has to be estimated (see below).

The key decisions are (from Gregory *et al.*, 2004):

- Do we want to estimate population size accurately or will an index meet our needs? In other words, are we interested in absolute or relative abundance (index)?
- Where will we undertake the survey?
- Should we cover the whole area of interest, or only sample part of it?
- If we plan to sample, how should we select the study sites?
- What geographical sampling units will we use? Mapped grid squares, forest blocks, or other parcels of land?
- What field method will we use?
- What are the recording units: individuals, singing males, breeding pairs, nests or territories?
- How will the subsequent data analysis be carried out?
- How will the results be reported and used?

You need to adapt your method to: a) the question you are asking, b) your skill, c) the time available, and d) the habitat. In open habitats, distance sampling may be easy and therefore the method of choice, but this will be hard in rainforest. Notice, though, that comparisons between habitats require that similar methods are being used.

When trying to survey a dense lowland rainforest you will quickly notice your limitations. It will be hard to see birds and to estimate distances to vocalizing birds. This makes standardised quantitative sampling difficult or even impossible (Bibby *et al.*, 2000). With the many logistic constraints during fieldwork in such environments, it is therefore important to consider how to best spend the time available. Rather than working hard to obtain perfect quantitative data from a single site, it may be better to use the time to get semi-quantitative data for several sites. This approximates random sampling of the metacommunity and, in addition, gives some information about variation across different habitats. Small samples mean that some rare species are unrecorded, and this truncation of the community (Preston, 1948) reduces the possibility to discriminate between different abundance models. However, even incomplete samples will suffice to identify dense (viable) populations of species of conservation concern, and will allow estimates of species richness.

3.1. Pilot Survey

Unless you have been there, you will not know what your survey area is really like. Plan a pilot survey of at least two weeks to a) get to know your species and the habitat, b) to try out methods and c) to practise them.

3.2. Qualitative methods, relative abundance

- **Simple species list.** All species are noted, regardless of time of day or season. The presence or absence of rare or threatened species (Birdlife International 2000, <http://www.iucnredlist.org/>) is the key for conservation and management of a site. The problem with simple species lists is that there is no control for observer effort. Chance observations will obviously increase with the time you spend in the field and some cryptic resident species don't show up immediately.
- **Species discovery curves.** Species discovery curves that record survey effort can be obtained by recording the time spent in the field for each observer. It is important that observers work at different areas or at different times. By also noting the date and time each species was discovered, some simple analysis becomes possible. Having separate lists for different areas may enable you to come up with further detail, e.g. if you split your area into degraded and natural forest, you can make a simple comparison
- **Encounter rates.** Encounter rates are calculated for each species by dividing the number of birds recorded by the number of hours spent searching, giving a figure of birds per hour for each species. When doing this separately for different habitats, more detailed information can be obtained. However, beware that encounter rates will vary with the structure of the vegetation. In dense vegetation encounter rates may be lower than in more open habitat. Encounter rates are not a substitute for true density estimates but they allow a comparison of relative abundance. Abundance categories can be scored.

As an example (from Lowen *et al.*, 1996), for each species assume the number of individuals/100 field hours to be your value, then you could use the following abundance categories: rare (< 0.1), uncommon (0.1-2.0), frequent (2.1-10.0), common (10.1-40.0) or abundant (>40.0). If these categories do not work for your data you can of course adapt them accordingly. To avoid counting birds several times, it is important that different observers move to a starting point at some distance from base camp and plan their routes so that they are not overlapping. Birds that call loudly will be recorded more often than more quiet ones and the likelihood to record a species will depend on its state in the annual cycle.

- **MacKinnon lists** (MacKinnon & Phillips, 1993). They are often used in "Rapid Assessments" (Herzog *et al.*, 2002). Make a list by recording each new species seen until you reach e.g. 20 species; then start again with a new list. Any one species will only be recorded once in your first list of 20, but may be recorded again in subsequent lists. Analysis of ten or more lists for a given area will give a good picture of its avifauna. Plotting the cumulative total number of species recorded against the number of lists made, this produces a species discovery curve whose steepness reflects species richness and indicates how many more species are likely to be found in an area (Fig. 2). If you are in a habitat that is species poor, you may decide to use a lower number than 20, maybe 15 or even 10 (Poulsen

et al., 1997). You need to try this out. Your speed of walking will greatly affect the kind of species you will encounter. Whilst walking slowly through the forest one will get a good number of scrub or canopy dwelling birds, but for some ground-dwelling birds such as pheasants, pittas or thrushes, moving quickly but silently through the forest will yield better results, as birds have less time to react to your approach. It is recommended to discover every bird that is active within 50 m from the transect line (Schieck, 1997) and thus it is not practically possible to achieve a constant walking speed.

If this method is used to describe community structure, there are some fundamental flaws, as some records will be single birds and others will be flocks with many individuals of the same species; fortunately this problem is not so serious in tropical forests where most species appear in pairs and family groups (2-4 individuals). The data can be much improved by writing down the number of individuals and use this raw data for the final analysis of community composition and species richness (Herzog *et al.*, 2002).

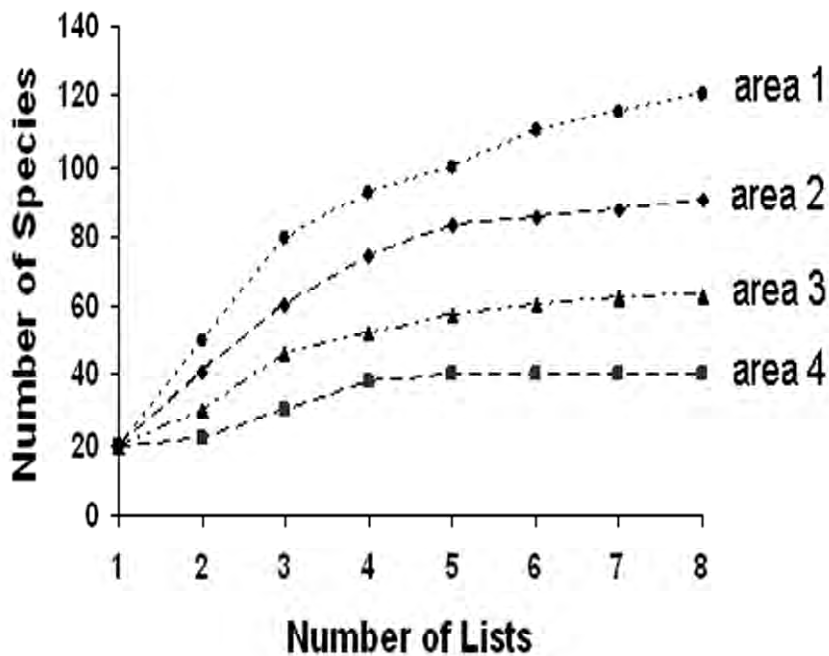


Fig. 2. Species curves derived from MacKinnon lists, simplified and altered from MacKinnon & Phillips (1993).

- Timed species counts.** Timed species counts (TSCs) are especially useful for open habitats (Pomeroy & Tengecho, 1986), but as mentioned above this approach may not be useful off-trail in thick vegetation. Data for TSCs are recorded in six columns, corresponding to six 10-min intervals during an hour-long survey. The observer walks at a slow pace (about 1-2 km/h). For the first 10 min, every species seen or heard is noted down in the first column, regardless of the number of individuals. For the second 10 min-

period, any species not already recorded is noted in the second column and so on. For one observation hour each species is only noted once. A minimum of 15 surveys should be carried out for a site, corresponding to 15 observation hours. Pomeroy & Tengecho (1986) suggest to physically cover an area of 1 km² for each count. Depending on the habitat this may of course be modified.

3.3. Quantitative Methods

- **Positioning of sampling points.** There are various possibilities to place sampling points or beginnings of transects (Fig. 3), each one with advantages and disadvantages. For statistical reasons it is important to place all sampling points at random or at least to place your first point in a line of points at random. When counting along roads or existing paths (Fig. 3A) it is likely that not all species are discovered because the path does not run through all the different habitats. More importantly, the presence of the road or path may influence the species (or numbers) present. Placing the points randomly may give better coverage (Fig. 3B), however, choosing a completely random approach may leave some areas unsampled. A stratified random sample (Fig. 3C) using a grid (at least 500 m apart) is the best choice. In each resulting square one point is chosen at random. If points from two neighbouring squares are too close to each other (so that possibly the same birds are counted twice) then it may be useful to omit that point and choose a new one.

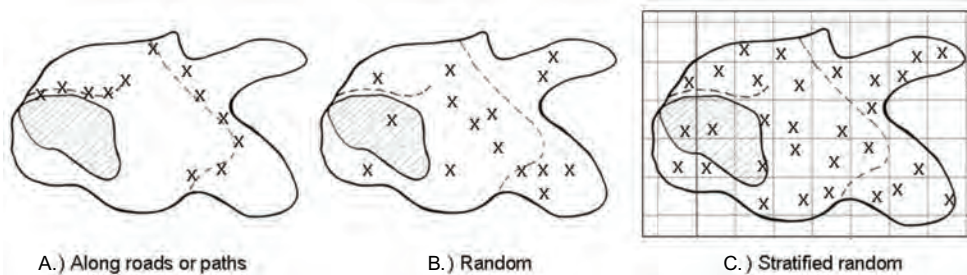


Fig. 3. Three possibilities for positioning point counts or beginnings of transects. a. along existing roads or paths; b. entirely random or c. stratified random (after Bibby *et al.*, 1998).

Method	What for?	Advantages	Disadvantages	Cost
Species list	Species present	Easy, no data analysis	No control of observer effort making comparison between areas or counts impossible	Cheap
Species discovery curve	Estimate of the total number of species present	Different sites and counts can be compared	Plotting of data requires computer analysis, but simple	
Encounter rate	Index of relative abundance for individuals of a species per unit time	Crude comparison of abundance between species within a site and within species between sites	Differences in species detectability not accounted for. To count all individuals of all species present can be a practical problem	
MacKinnon lists	Index of relative abundance based on the number of encounters with species per block of effort. Plotting a species discovery curve	Crude comparison of abundance between species within a site and within species between sites. Data collection is simple, allowing the observer freedom to roam. Relatively unaffected by observer skill and concentration	Differences in species detectability not accounted for, underestimation of flocking species	
Timed species-counts	Index of relative abundance based on the number of encounters with species per weighted block of time. Plotting a species discovery curve	Crude comparison of abundance between species within a site and within species between sites. Data collection is fairly simple, allowing the observer freedom to roam	Underestimation of flocking species	
Mist-netting	Secret understorey species, index of relative abundance when use of standardized net length and time	Detect understorey birds, get to know the birds	Proper training and special equipment required, time-consuming, mostly limited to understorey species, not cost-effective, capture conditions introduce strong bias	

Table 1. Bird survey and count methods (adapted from Bibby *et al.*, 1998).

There are some practical considerations for choosing a certain sampling pattern and distance between sampling units (Robinson *et al.*, 2000). In a mountainous rainforest with dense understorey using existing paths or roads may be the method of choice because: a) observers could get lost otherwise, b) finding random points would be very time consuming and c) to get to these points a lot of vegetation needs to be cut down which is not only time consuming but may also be quite destructive. Furthermore, some canopy species are impossible to detect when obscured by foliage, thus using a road may enable you to see them (MacKinnon & Phillips, 1993). When cutting transects during the breeding season there is a danger to destroy nests and you will open pristine forest to people and animals that may follow your tracks to exploit the forest. The disadvantage is obvious – you will not cover your study area evenly, thereby not encountering some bird species that you may have found using a random approach. In forest, two sampling points should be at least 150 to 200 m apart, in open habitat even further. Doing your survey in open farmland savannah it will be more easy to set up transects or to find random points.

- **Distance sampling** (from Bibby *et al.*, 1998). Quantitative methods often require the estimation of the distance between the observer and the bird (Fig. 4). Errors can be minimized by practising beforehand, and it is important that different team members synchronize their estimates. Optical range finders can be useful when you see a bird (but not when you hear it). If you sample from points you can mark certain distances in advance. However, in dense vegetation neither of these methods will work. Estimation of the distance to a calling bird can be practised by placing a tape recorder at various distances. To make things easier one can use distance bands, *i.e.* within 5, 10, 15, 20, 30, 40, 60, 80, 100 m of the observer.

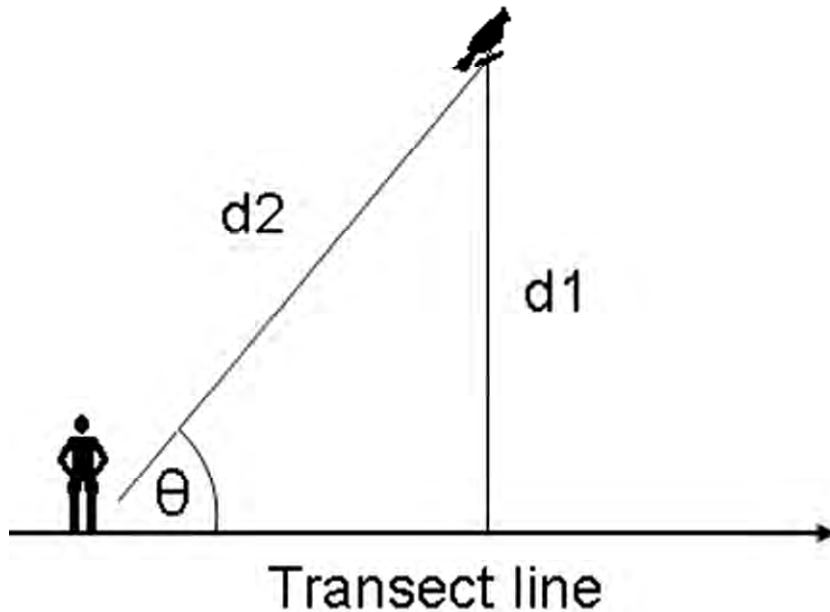


Fig. 4. d_1 (perpendicular distance) can be calculated using d_2 and the sighting angle θ ($d_1 = d_2 \cdot \sin(\theta)$).

For the analysis of these data the software “Distance” can be used (Laake *et al.*, 1994). Please check Bibby *et al.* (1998) for further detail. Distance sampling can be used both in line counts and point counts (see Table 2 for advantages of each method).

- **Point Counts.** Points are usually laid out on a random transect, *i.e.* every 50 m. One walks from one point to the other, stops at the point for a predetermined amount of time (*i.e.* 5-10 min) to count all birds present (individuals and species) and then walks to the next point to repeat this and so forth. Distance between points and amount of time spent counting need to be adapted to the habitat. For more details see Bibby *et al.* (1998).
- **Line Counts.** You walk continuously along a certain line and record all bird contacts either side of the track. Walking speed should be constant, a goal very hard to achieve, especially in dense forest. Avoid counts along streams and rivers as your splashing about will flush birds along the river often long before you have even had a glimpse on them. For more details see Bibby *et al.* (1998).

Line Counts	Point Counts
+++ extensive, open and uniform habitats	+++ dense forest or scrub
+++ mobile, large or conspicuous species and those that easily flush	+++ cryptic, shy, skulking species
+++ low population densities or species poor	+++ high population densities or species rich
Cover the ground quickly and efficiently recording many birds	Time is lost whilst walking between points, but at point more time for observation and identification
Double counting minor as observer is on the move	Double counting potential problem
Birds are not so much attracted to observer	Curious birds may be attracted to the observer
+++ when easy access	+++ when difficult access
Can be used for bird-habitat studies	Better suited to bird habitat studies
Errors in distance estimation have a smaller influence on density estimates (because the area sampled increases linearly from the transect line)	Errors in distance estimation can have a larger influence on density estimates (because the area sampled increases geometrically from the sampling point)

Table 2. Differences between point and line counts (modified from Gregory *et al.*, 2004), +++ = good for.

- Territory mapping and other methods. For more advanced methods like territory mapping we refer to the literature (i.e. Bibby *et al.*, 2000). A method especially designed for tropical environments is “Multi Time-Window Transect-Mapping” (see Jahn, in press).

3.4. Mist-netting

Mist-netting is useful to learn the birds in an area and to discover skulking understorey species but it is not time effective and the data obtained are not useful for a quantitative description of the local bird community. According to Remsen & Good (1996), the record of birds through mist-netting measures the activity of birds within 3-4 m above the ground rather than the community composition in the habitat. Mist-netting is very time-consuming, extremely weather dependant and should only be carried out by well-trained people. A good description of how to responsibly use mist nets to catch birds is given in Redfern & Clark (2001). Training is offered in many countries by institutions that organize the national bird ringing schemes.

Europe: http://www.euring.org/national_schemes/contact_schemes.htm

Out Europe:

http://www.euring.org/national_schemes/non_euring_schemes.htm.



Fig. 5. A mist-net of 6 m height using bamboo poles to catch birds in Madagascar. (Photo: F. Woog).

Also see Hofmann *et al.* (this manual) for a detailed description of the use of mist-nets for catching bats. For most passerines, 16 mm Nylon mesh is used, nets are 6 or 12 m long and have 4-5 shelves (giving them a height of about 2.5 m). Mistnets can be placed on ridges, in thickets or at forest edges, where birds often pass close to the ground. If nets are set in wrong places a lot of damage can be done to the birds (bird colonies, roosting places and nests have special rules). Generally, avoid putting the nets in the sun, where they are easily visible, and where captured birds can rapidly dehydrate. Taking birds out of the nets, and handling them, has to be done properly and enough people need to be present to monitor the nets that have been put up at close intervals (every hour, or more often under warm conditions or when it drizzles; but close the nets when it rains!). After extraction from the net birds are usually placed in double-sowed light cotton bags for further processing.

Birds can transmit diseases. To reduce this risk, make sure that bird bags stay dry and clean (turn them inside out and shake them after each capture, and wash them often). Make sure to thoroughly disinfect nets, especially when moving between countries or continents. It is unacceptable to use mist-nets first in an European country and then use them, unwashed, in a pristine tropical forest (the same is of course true for all your camping and outdoor equipment). Also observe principles of hygiene: wash your hands, preferably with disinfective soap. Do not take soiled bird bags into the tent you sleep in.

When you have the bird in your hand, make careful records (photos, weight, fat score, brood patch, moult stage, wing length and other measurements) (Svensson, 1992; Baker, 1993) and, depending on additional research questions, take a blood sample for DNA-analysis or feather for the analyses of stable isotopes (see paragraph on collecting birds). Bird ectoparasites are often poorly known and collecting them may yield new species (preserved in 2 ml vials in 70% alcohol, use very fine tweezers).

Canopy nets can increase the number of species you catch. They are not easy to handle. The best way to learn about these is to ask somebody that uses them.

To quantify birds, mist-netting is not really a good method and will only be useful to compare relative abundances of selected understorey species (see Table 1). If this is planned, the birds need to be marked to avoid double counting recaptures as new captures. The most useful are bird rings, but if these are not available tail feathers can be clipped systematically to identify birds which have already been caught. Clippings should be as small as possible. As birds moult their feathers at least annually, these markings remain temporary. Mark-recapture methods are useful for a wide variety of studies and purposes and can help to estimate abundance of selected species quite precisely or establish local movement pattern (for information about data analysis of capture-recapture data see <http://warnercnr.colostate.edu/~gwhite/mark/mark.htm>).

A note of caution – locals often ask for mist-nets (as a means of catching birds for food, plumage or for the pet-trade). Never leave mist-nets unattended. When storing them make sure they are as safe as your money and passport. On the other hand, some locals may be afraid of the nets and will not pass by. Make sure you talk to the village people that may encounter the nets, ask them for their permission and explain what you are doing. In some areas with large game, goats, cows or monkeys it may not be advisable to use mist-nets as these animals can easily destroy your expensive nets and may severely injure themselves during the process of entanglement.

3.5. Collecting birds

For a bird survey and monitoring scheme the collection of birds is usually not necessary, but sometimes new species remain undiscovered because birds were not collected. There are all sorts of opinions about collecting birds (see Remsen, 1995; Collar, 2000). Habitat loss, agricultural practice and world-wide climate warming are the real threats to birds, and in comparison the “sacrificing” of a few birds for science means nothing for most species except when they are very rare. But all collecting should be done legally and justified by some clear purpose, such as needs for documenting new-discovered populations and potential new taxa. In many developing countries there exists a sort of split moral – one for indigenous people, one for industrial enterprises and one for (foreign) biologists. Whereas indigenous people hunt birds for a living, and international companies destroy vast pristine natural areas (*i.e.* for mining, dam projects etc.) often, especially foreign, biologists are not being granted collection and export permits for birds. Even the collection of feathers and birds found

dead along roadsides may not be permitted and the export from the country of origin and import into your home country is not easy (*i.e.* concerning species listed under CITES or under health regulations).

However, it is always useful to prepare birds found dead or for a specific scientific question, and local partners can advise on what to do concerning permits. The easiest is usually a cooperation with a museum or university where specimens can be kept before permits have been worked out. It is fair enough that these institutions often want a share of the collected material for their own collections. For preparation techniques see Wagstaffe & Fidler (1968), Harrison & Cowle (1970), Piechocki (1998), Winker (2000) and Hofmann *et al.* (this volume). To see a video on bird preparation, paste <mms://137.229.54.15/bts/birdprep.wmv> into your browser.

In order to preserve bird skins under field conditions it is best to prepare the skin right away, and to quickly dry it properly (*i.e.* using a kerosene lamp for heating, when it rains). Remove the brain and as much tissue as possible. Salt can be used as a cheap and easily available preservative agent. Thymol can help to prevent bacterial growth and moulding, *i.e.* put some crystals into your air-tight storage containers, and if needed silica gel. If one has no time to make skins in the field or is working in climates with a high humidity, birds can be put into 70 % alcohol (1/3 animal, 2/3 alcohol). When preserving a complete animal without skinning it you need to inject alcohol with a syringe into the internal cavity of the animal and the brain (through the nose). Because the alcohol gets diluted by the fluids of the animal, it is advisable to change it after a few days. Alcohol can wash out certain colours, which is a disadvantage. For transport, the alcohol can be drained and specimens be put into double Ziplock bags. It is also useful to collect a tissue sample (*i.e.* muscle or liver) in the field. This is to be stored in pure 90-95% alcohol or EDTA-buffer. For good practices in tissue conservation: http://www.mip.berkeley.edu/mvz/collections/opportunistic_collection_of_tissue.pdf.

For many research purposes it may suffice to take a blood or feather sample and then releasing the bird (note however, that official permits may still be needed!). Blood samples are useful for genetic studies on various levels not only for speciation but also for population differences (Gaunt, 1999; Dawson, 2005). Stable isotopes found in feathers can give you an indication where a migratory bird grew a feather (Bearhop *et al.*, 2000; Wassenaar & Hobson, 2001). A small drop of blood is taken from the wing vein or in species with soft legs (like swans, geese and ducks) or young birds from the leg vein and placed in small vials containing buffer (200-300 µl blood in storage buffer containing 10% EDTA, 1% SDS, 0.5% NaF, 0.5% thymol and 100 mM Tris, pH 7.4 (Wink, 2006) or alternatively DMSO-buffer (SSDE) consisting of 20% DMSO, 0.25M EDTA pH 8.0 saturated with NaCl). These samples can be stored at ambient temperatures, but longer-term storage at -20°C (or lower) is recommended. Care should be taken, that syringes or buffer do not contain heparin, as this will inhibit the PCR reactions. The procedure should be learned from another ornithologist that has used the method before. Veterinarians often do not have experience extracting blood from birds.

4. Documentation

4.1. Labels

When collecting samples make sure they are properly labelled, a collection number is not enough. A proper label should at minimum contain date (write out the month *i.e.* 11 Dec 2009, not 11.12.2009 as this may be read 11/12/2009 which could be interpreted as 12 Nov 2009, always write the complete year as 09 could mean 1909, 1809 or 2009), exact location including country, species, collector, collection number. Never trust that you will remember to do this later.

<http://olla.berkeley.edu/ornisnet/?q=node/5> gives detailed tools and guidelines for geo-referencing. If possible, note longitude and latitude (*i.e.* read from your GPS or map).

4.2. Proper documentation

Field notes should always be detailed, with date, time of day, weather observation (that may influence your survey results, *e.g.* heavy rainfall) and, if possible, number of individuals encountered, and, if discernible, their sex and age. If you see a group of peacocks, for example, note number of males, females, immatures and juveniles. If you encounter birds that you do not know, try to take a photograph, take a sound recording or immediately make a small sketch of what it looked like. If you don't have enough time in the field or you can't write things down whilst you are observing birds, MP3 players can serve as dictaphone. However, always think about the time it will take to transcribe the information from your recordings. One can only guess how many recordings have been made in ornithological research without ever having been analyzed. Modern digital cameras offer a unique possibility for improving field identification. For instance, when a mixed feeding party of birds passes through the vegetation, take as many photographs as you can, and by zooming in afterwards you can identify birds that you did not immediately have time to identify (or later blow up the images on your computer screen). In this way you may sometimes be able to reliably identify every bird in the party.

It is often useful to enter data in forms prepared in advance, as this may facilitate later data entry into your database. The forms should mirror the structure of the database you intend to use. Enter your data as quickly as possible.

When working in wet climates, working with normal paper is a challenge as at the end of your expedition you may end up with a heap of 'papier mâché'. Fortunately there are solutions, *i.e.* "Write in the Rain" notebooks and copying paper (Darling Corporation, <http://www.riteintherain.com>). These items are not cheap but are well worth the investment. If you use pencil you can drop them in a river and will still be able to read what you have written.

4.3. Database and data analysis

This should be in place before you start the survey, as they are very much linked. Good guidance can be found in Bibby *et al.* (1998, 2000) and Voříšek *et al.* (2008). The statistical approaches for estimating bird abundance from bird counts and taking detectability into account (*i.e.* Kéry, 2008) are well beyond the scope of this chapter. Whenever in doubt, consult a professional ornithologist or statistician before you start fieldwork.

5. Case studies

5.1. Case study 1 from a tropical cloud forest (The Chelemhá, Guatemala)

Combined line-point counts are often used in the tropics. Almost any method is biased to sample the entire bird community (Terborgh *et al.*, 1990; Poulsen, 1994; Remsen, 1994; Remsen & Good, 1996). Therefore, a combination of several methods is sometimes essential to get a complete species list and estimate relative abundance. In Guatemala, it proved essential to combine point counts with transects, since a large part of the bird community would have been missed if using only one method (Renner, 2003). To circumvent losing some essential species, point counts were combined with transects counts: point count sites were established each 25 m along transects. At each 25 m mark, all birds sighted or heard within a nominal distance of 100 m were recorded for five minutes. After the five minutes, the distance to the next point count locality was slowly followed in the shortest possible way covering the distance in about the same time. Transects were 150 m apart totalling 3,300 m. The local cloud forests, the major habitat in Chelemhá, fortunately do not have a very dense understorey, hence only minor efforts were needed to establish the point-transects. The bordering secondary vegetation, however, was very dense, and establishing trails to count birds was impossible (the dense secondary vegetation was a wall consisting of 2 cm thick stems of plants only 20 cm apart at the time of monitoring). The Guatemalan authorities and the land owner were interested in the results of the bird survey but because the area was a non-use forest reserve, all cutting of vegetation was banned. The establishment of point sites and transects was therefore a trade-off between scientific desire (random) and conservation (using existing trails as much as possible and minimizing the impact on the area). To diminish effects of detectability (Hines, 2006; MacKenzie *et al.*, 2002, 2003), all point counts/transects were visited three times a year. The data were used to establish relative abundance of all bird species and to determine presence of species (Magurran, 1988; Rosenzweig, 1995). Results showed that while more species were present in secondary forest, all species of conservation concern were only present in natural forest (Renner, 2003, 2005).

5.2. Case study 2 from cloud forests in the mountains of the tropical Andes

Because of the steepness of the terrain, impenetrable vegetation and lack of trails it was difficult to standardise the sampling and to obtain reliable bird density data (Bibby *et al.*, 2000). Rather than trying to get absolute quantitative data from one or two study plots, the study aimed to obtain semi-quantitative data for comparing the community composition of samples over several sites and habitats.

Avian community data were obtained during transect walks. All visual and acoustical records of birds within 50 m (Schieck, 1997) were noted while walking very slowly and quietly through the terrain and as "randomly" (with frequent changes in direction) as topography and vegetation permitted (Fjelds , 1999; Herzog *et al.*, 2002). Species accumulation curves level out rapidly (much more so than with point sampling!) and high correlations between relative species abundance data obtained this way and by point-counts in the same area suggests that observations made during "random" walks are not significantly more biased than those obtained by more standardised point counts (Fjelds , 1999). The main advantages of this "random-walk" approach are the broad sampling of the study area, time-efficiency (all bird observations being used, unlike in point counts; see below) and relative observer independence (Sauer *et al.*, 1994) compared to timed-species-count methods.

At each study site, data were collected within 1-1½ km² and over 2-4 days. Variation in the extent of study plots is not of a magnitude that requires adjustment for area differences. Study sites of this size will represent habitat mosaics (of different associations of forest trees, tree-fall gaps, landslides and glades) but the study plot was large enough to find most birds on the move, singly or in mixed feeding parties. Walking speed varied (as the vegetation is sometimes nearly impenetrable) but was usually ca. 500 m per hour. On average, 0.4 birds were identified per minute. This rate could be raised by walking faster, but the data will then be more biased towards easily detectable species.

The observed species richness is constrained by sample size, and for comparison it is necessary to estimate species richness by extrapolation. Such estimators reach their own asymptote much sooner than sample-based rarefaction curves, they level off and approximate empirical asymptotes well.

The simplest approach (which can be applied currently, during field work) is to use the Chao 1 formula (Colwell & Coddington, 1994):

$S1 = Sobs + a/2b$ (*Sobs* being the number of species recorded, *a* being the number of singletons = number of species recorded only once, *b* the number of doubletons).

A more sophisticated estimation can be done later using Colwell's software EstimateS (<http://viceroy.eeb.uconn.edu/estimates>). Ranked abundance curves can be constructed from the total list of observed birds along the route, assuming that the attentive observer is able to detect all birds (at least those which are active) within 50 m from a transect. In most cases, about 500 bird

identifications will provide a good sample for describing the bird community within a study plot.

John MacKinnon and Karen Phillips on birdwatching in forest:..." *Watching birds in tall forests is not easy. You may walk for an hour without seeing anything then suddenly be surrounded by so many twittering birds that you cannot focus on any. A bird may be so high up and so obscured by foliage that you cannot get a good view. In the rain, water on your lenses may blur your vision... leeches are an accepted irritation*"...

6. Acknowledgements

We do sincerely thank Michel Louette (Royal Museum for Central Africa, Tervuren, Belgium), Dieter Oschadleus (Animal Demography Unit, University of Cape Town, South Africa), Olaf Jahn (Aves & Conservación, Corporación Ornitológica del Ecuador), Richard Gregory (RSPB, England, EBCC), Andy Musgrove and David Noble (BTO, Thetford, England) and Hans-Günther Bauer (Max-Planck Institute for Ornithology and EBCC, Radolfzell, Germany) for helpful discussions, ideas and comments on earlier drafts of this manuscript.

7. References

7.1. Reference List

AMERICAN ACADEMY OF ORTHOPAEDIC SURGEONS (AAOS) 2008. *Wilderness First Aid*. Jones and Bartlett Publishers. Boston, Toronto, London, Singapore: 369 pp.

BAKER, K. 1993. *Identification Guide to European Non-Passerines*. BTO Guide 24. Thetford: 332 pp.

BEARHOP, S., PHILLIPS, R.W., THOMPSON, D.R., WALDRON, S. & FURNESS, R.W. 2000. Variability in mercury concentrations of Great Skuas *Catharacta skua*: the influence of colony, diet and trophic status inferred from stable isotope signatures. *Marine Ecology Progress Series* 195: 261-268.

BIBBY, C., JONES, M. & MARSDEN, S. 1998. *Expedition Field techniques: Bird Surveys*. Royal Geographical Society, London. [Online], Available: <http://biology.kenyon.edu/courses/biol229/fieldmanual%20birds.pdf> [2009, Dec].

BIBBY, C.J., BURGESS, N.D., HILL, D.A. & MUSTOE, S.H. 2000. *Bird Census Techniques*, 2nd edition, Academic Press, London: 277pp.

BIRDLIFE INTERNATIONAL 2000. *Threatened Birds of the world*. Lynx Edicions and Birdlife International, Barcelona and Cambridge 852 pp.

BORROW, N. & DEMEY, R. 2004. *Birds of Western Africa*. Helm Field Guides, London: 551 pp.

COLLAR, N. 2000. Opinion. Collecting and conservation: cause and effect. *Bird Conservation International* 10(1): 1-15.

- COLWELL, R.K. & CODDINGTON, J.A. 1994. Estimating terrestrial biodiversity through extrapolation. *Philosophical Transactions of the Royal Society (Series B)* 345: 101-118.
- DANIELSEN, F., BURGESS, N.D. & BALMFORD, A. 2005. Monitoring matters: examining the potential of locally-based approaches. *Biodiversity and Conservation* 14: 2507-2542.
- DANIELSEN, F., BURGESS, N.D., BALMFORD, A., FJELDSA, J., ANDRIANANDRASANA, H.T., BECKER, C.D., BENNUM, L., BRASHARES, J.S., CHRISTIANSEN, S., DONALD, P.G., EGUINO, S., ENGHOFF, M., FUNDER, M., GRAY, M., HÜBERTZ, H., JONES, J.P.G., OETTING, I., POULSEN, M.K., RIJSOORT, J., STUART-HILL, G., TOPP-JØRGENSEN, E., TOWNSEND, W.R., UYCHIAOCO, A.J., WHITTEN, T. & YONTEN, D. 2006. Monitoring matters: evaluating locally-based biodiversity monitoring in developing countries. *Oryx* 40: 14-15.
- DANIELSEN, F., MENDOZA, M.M., TAGTAG, A., ALVIOLA, P.A., BALETE, D.S., JENSEN, A.E., ENGHOFF, M. & POULSEN, M.K. 2007. Increasing conservation management action by involving local people in natural resource monitoring. *Ambio* 36: 566-570.
- DAWSON, A. 2005. Techniques in physiology and genetics. In : Sutherland, W.J., Newton, I. & Green R.E. (Eds). *Bird Ecology and Conservation. A Handbook of Techniques*. Oxford University Press, Oxford: 211-231.
- FJELDSA, J. 1999. The impact of human forest disturbance on the endemic avifauna of the Udzungwa mountains, Tanzania. *Bird Conservation International* 9: 47-62.
- GAUNT, A.S., ORING, L.W., ABLE, K.P., ANDERSON, D.W., BAPTISTA, L.F., BARLOW, J.C. & WINGFIELD, J.C. 1999. *Guidelines to the use of wild birds in research*. The Ornithological Council, Washington DC: 56 pp.
- GIBBONS, D.W. & GREGORY, R.D. 2006. Birds. In : Sutherland W.J. (Ed.). *Ecological Census Techniques*, 2nd Edition. Cambridge University Press, Cambridge: 308-344.
- GILBERT, G., GIBBONS, D.W. & EVANS, J. 1998. *Bird Monitoring Methods: a manual of techniques for key UK species*. RSPB, Sandy: 464 pp.
- GREGORY R.D, GIBBONS, D.W & DONALD, P.F. 2004. 'Bird census and survey techniques'. In : Sutherland, W.J., Newton, I. & Green R.E. (Eds). *Bird Ecology and Conservation. A Handbook of Techniques*. Oxford University Press, Oxford: 17-55.
- HARRISON, C.J. & COWLES, G.S. 1970. *Birds: Instructions for Collectors*. 1st edition. British Museum, London: 48 pp.
- HERZOG, S.K, KESSLER, M. & CAHILL, T.M. 2002. Estimating species richness of tropical bird communities from rapid assessment data. *The Auk* 119: 749-769.
- HINES, J.E. 2006. *PRESENCE2- Software to estimate patch occupancy and related parameters*. United States Geological Survey–Patuxent Wildlife Research Center (USGS-PWRC). [Online], Available: <http://www.mbr-pwrc.usgs.gov/software/presence.html> [2009, Dec].

- JAHN, O. in press. Bird communities of the Ecuadorian Chocó: a case study in conservation. *Bonner Zoologische Monographien* 56.
- KÉRY, M. 2008. Estimating abundance from bird counts: Binomial Mixture Models uncover complex covariate relationships. *The Auk* 125(2): 336-345.
- LAAKE, J.L., BUCKLAND, S.T., ANDERSON, D.R. & BURNHAM, K.P. 1994. *Distance User's guide Version 2.1*. Colorado Cooperative Fish and Wildlife Research Unit, Colorado State University, Fort Collins: 84 pp.
- LOWEN, J.C., BARTRINA, L., CLAY, R. & TOBIAS, J. 1996. *Biological surveys and conservation priorities in eastern Paraguay*. CSB Conservation Publications, Cambridge: 186 pp.
- MACKENZIE, D.I., NICHOLS, J.D., LACHMAN, G.B., DROEGE, S., ROYLE, J.A. & LANGTIMM, C.A. 2002. Estimating site occupancy rates when detection probabilities are less than one. *Ecology* 83: 2248-2255.
- MACKENZIE, D.I., NICHOLS, J.D., HINES, J.E., KNUTSON, M.G. & FRANKLIN, A.B. 2003. Estimating site occupancy, colonization and local extinction when a species is detected imperfectly. *Ecology* 84: 2200-2207.
- MACKINNON, J. & PHILLIPS, K. 1993. *A Field Guide to the Birds of Sumatra, Java and Bali*. Oxford University Press, Oxford: 491 pp.
- MAGURRAN, A.E. 1988. *Ecological diversity and its measurement*. Princeton University Press, Princeton: 179 pp.
- MERRY, W. 1994. *The Official Wilderness First Aid Guide*. McClelland & Stewart, Toronto: 408 pp.
- NG'WENO, F. 2008. Pitfalls in ethno-ornithological research. In : Harebottle D.M., Craig A.J.F.K., Anderson M.D., Rakotomanana H. & Muchai M. (Eds). *Proceedings of the 12th Pan-African Ornithological Congress*. Animal Demography Unit, Cape Town: 44-45.
- PIECHOCKI, R. & ALTNER, H.-J. 1998. *Makroskopische Präparationstechnik. Teil I, Wirbeltiere*. Verlag Gustav Fischer, Jena, Stuttgart, Lübeck, Ulm: 461 pp.
- POMEROY, D. & TENGECHO, B. 1986. Studies of birds in a semi-arid area of Kenya. III – The use of “timed species-counts” for studying regional avifaunas. *Journal of Tropical Ecology* 2: 231-247.
- POULSEN, B.O. 1994. Mist-netting as a census method for determining species richness and abundances in an Andean cloud forest bird community. *Le Gerfaut* 84: 39-49.
- POULSEN, B.O. 1996. Structure, dynamics and home-range of two mixed species flocks and species composition in a montane Alder dominated secondary forest in Ecuador. *Journal of Avian Biology* 12: 333-343.
- POULSEN, B.O., KRABBE, N., FRØLANDER, A., HINOJOSA, M.B., & QUIROGA, C.I. 1997. A rapid assessment of Bolivian and Ecuadorian montane avifaunas using 20-species lists: efficiency, biases and data gathered. *Bird Conservation International* 7: 53-67.

- PRESTON, F.W. 1948. The commonness and rarity of species. *Ecology* 29(3): 254-283.
- RASMUSSEN, P.C. & ANDERTON, J.C. 2005. *Birds of South Asia. The Ripley Guide*. Vols. 1 and 2. Smithsonian Institution and Lynx Edicions, Washington, D.C. and Barcelona: 378 & 685 pp.
- REDFERN, P.F. & CLARK, J.A. 2007. *Ringer's manual*. BTO, Thetford: 270 pp.
- REMSEN, J.V. 1994. Use and misuse of bird lists in community ecology and conservation. *The Auk* 111: 225-227.
- REMSEN, J.V. 1995. The importance of continued collecting of bird specimens to ornithology and conservation. *Bird Conservation International* 5: 145-180.
- REMSEN, J.V. & GOOD, D.A. 1996. Misuse of data from mist-net captures to assess relative abundance in bird populations. *The Auk* 113: 381-398.
- RENNER, S.C. 2005. The Resplendent Quetzal (*Pharomachrus mocinno*) in the Sierra Yalijux, Alta Verapaz, Guatemala. *Journal of Ornithology* 146:79-84.
- RENNER, S.C. 2003. *Structure and diversity of cloud forest bird communities in Alta Verapaz, Guatemala, and implications for conservation*. Niedersächsische Staats- und Universitätsbibliothek Göttingen. [Online], Available: <http://webdoc.sub.gwdg.de/diss/2003/renner/renner.pdf> [2009, Dec].
- ROBERTS, D.L., COOPER, R.J. & PETIT, L.J. 2000. Flock characteristics of ant-following birds in premontane moist forest and coffee agroecosystems. *Ecological Applications* 10(5): 1414-1425.
- ROBERTSON, H. & HEATHER, B. 2004. *The Hand Guide to the Birds of New Zealand*. Oxford University Press, London: 168 pp.
- ROBINSON, W.D., BRAUN, J.D. & ROBINSON, S.K. 2000. Forest bird community structure in central Panama: influence of spatial scale and biogeography. *Ecological Monographs* 70: 209-235.
- ROSENZWEIG, M.L. 1995. *Species diversity in space and time*. Cambridge University Press, Cambridge: 436 pp.
- SAUER, J.R., PETERJOHN, B.G. & LINK, W.A. 1994. Observer differences in the North American Breeding Bird Survey. *The Auk* 111: 50-62.
- SCHIECK, J. 1997. Biased detection of bird vocalizations affects comparisons of bird abundance among forested habitats. *Condor* 99: 179-190.
- SVENSSON, L. 1992. *Identification Guide to European Passerines*. 4th edition, revised and enlarged, self-published, Stockholm: 367 pp.
- TERBORGH, J., ROBINSON, S.K., PARKER, T.A., MUNN, C. & PIERPONT, N. 1990. Structure and organization of an Amazon forest bird community. *Ecological Monographs* 60: 213-238.
- VALLELY, A.C. 2001. Foraging at army ant swarms by fifty bird species in the highlands of Costa Rica. *Ornitologia Neotropical* 12: 271-275.

VOŘÍŠEK, P., KLVAŇOVÁ, A., WOTTON, S. & GREGORY, R.D. 2008. *A Best Practice Guide For Wild Bird Monitoring Schemes*. Royal Society for the protection of Birds, Sandy: 150 pp.

WAGSTAFFE, R. & FIDLER, J.H. 1968. *Preservation of Natural History Specimens*. Littlehampton Book Services Ltd., vol.2, part 2: Zoology: Vertebrates. Witherby, Philosophical Library of London: 115 pp.

WASSENAAR, L.I. & HOBSON, K.A. 2001. A stable-isotope approach to delineate catchment areas of avian migration monitoring stations in North America. *Environmental Science and Technology* 35: 1845-1850.

WERNER, D. 1979. *Where there is no doctor. A village health care handbook for Africa*. McMillan, Oxford: 440 pp.

WINK, M. 2006. Use of DNA markers to study bird migration. *Journal of Ornithology* 147: 234-44.

WINKER, K. 2000. Obtaining, preserving, and preparing birds. *Journal of Field Ornithology* 71: 250-297.

7.2. Useful Internet links

Birdlife International.

<http://www.birdlife.org/regional/index.html>

Sound archives.

<http://www.bl.uk/soundarchive>

<http://www.birds.cornell.edu>

http://www.xeno-canto.org/africa/index_static.html

http://www.xenocanto.org/index_static.html

http://www.xeno-canto.org/asia/index_static.html

http://www.xenocanto.org/australasia/index_static.html

Bird collections.

<http://www.museum.lsu.edu/~Remsen/AVECOLlections.html>

http://www.scricciolo.com/European_Bird_Collections_C%20S%20Roselaar.pdf

<http://olla.berkeley.edu/ornisnet/> (American Bird collections, ORNIS)

Bird species.

<http://avibase.bsc-eoc.org/avibase.jsp>

Online access to bird collection data.

<http://www.gbif.org>, <http://neotropical.birds.cornell.edu/portal/home>

http://www.zmuc.dk/VerWeb/Tanzanian_Vertebrates/TanzVert.index.html

Bird monitoring.

<http://monitoringmatters.org>

South-African Bird Atlas.

<http://sabap2.adu.org.za/index.php>

Guidelines to the use of wild birds in research.

http://www.nmnh.si.edu/BIRDNET/GuideToUse/Guidelines_2d_edition.pdf

European Bird census council.

<http://www.ebcc.info/index.php?ID=365>

Waterproof paper.

<http://www.riteintherain.com> (Darling Corporation)

Tissue collection.

http://www.mip.berkeley.edu/mvz/collections/opportunistic_collection_of_tissue.pdf

Bird preparation.

<http://www.uaf.edu/museum/bird/personnel/KWinker/Winker%20specimen%20preparation%20J%20Field%20Ornithol%202000.pdf>

<http://www.springerlink.com/content/100125/>

Data analysis of capture-recapture data.

<http://warnercnr.colostate.edu/~gwhite/mark/mark.htm>

7.3. Books

Voříšek *et al.*, 2008. A Best Practice Guide for Wild Bird Monitoring Schemes.
<http://www.ebcc.info/index.php?ID=365>

Sutherland *et al.*, 2004. Ecological Census Techniques, see section on “Gratis Book Scheme”.

http://assets.cambridge.org/97805218/44628/frontmatter/9780521844628_frontmatter.

Chapter 22

Preservation of freshwater fishes in the field

by

Dirk Neumann

Zoologische Staatssammlung München
Section Ichthyology / DNA-Laboratory
Münchhausenstr. 21, 81247 Munich, Germany
Email: Dirk.Neumann@zsm.mwn.de

Abstract

Collecting in rivers, streams or lakes is challenging, and the fishing efficiency is highly depending on habitat conditions and the selection of fishing gear. While some fishing gear can be very targeted, most gear types result in the capture of non-specific by-catch. Therefore, careful and thorough planning of any field project is essential in ensuring the collection of undamaged, well preserved samples, including the targeted species. Planning not only includes preparatory work before heading into the field such as applying for permits and the selection of suitable fishing gear for the specific habitat and species to be sampled, but also putting together comprehensive sampling equipment for the activities to be undertaken and assembling adequate personnel to handle all sampling events during the specified time period.

Key words: Fishing gear, fishing methods, fishes, preservation, tissue sampling

1. Introduction

“If field workers understand how specimens are processed and used in museums, they will prepare better specimens. 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). Besides your own research interests, specimens in natural history collections serve as valuable representatives of natural populations for other scientists for decades or even centuries. Therefore, all collections from natural (fish) populations should be made careful and with the best preservation procedures to ensure the highest quality of the preserved specimens and tissues for future research. Collection of ancillary material (additional specimens, species or amount of tissue) should be evaluated against the time, effort and money invested in the sampling. With collecting becoming increasingly difficult both due to budget and permitting issues, collaborative collecting is becoming more prevalent and cost effective.

2. Permits, regulations and responsibilities

Ecologists and Biologists working with freshwater fishes have to cope with many regulations and obligations. Besides national and international regulations and provisions on species covered under the *Convention on International Trade in Endangered Species of Wild Fauna and Flora* (CITES), additional permits and licences are necessary or required for endangered, threatened or protected species and have to be considered in the planning of a proposed field work project. These should not only include the required permitting legislation, but also consider the ethical treatment of the collected specimens. Minimizing actions and conditions that might induce physiological stress, physical damage and injury are not only a matter of animal welfare, but may have a direct impact on the quality of the preserved fishes in the field.

2.1. Collecting permits

Prior to the start of any field programme and depending on which country the field work is to be conducted in, all necessary permits for the proposed fishing and sampling activities in the study area must be obtained. This includes official research permits on multiple national/federal levels, fishing permissions, a valid fishing licence at least for the fishing person (mandatory in Europe and North America) and especially local permits (*i.e.* allowance/authorisation of local fishing right holders, communities, village chiefs, etc.). For entry and collecting in several National Parks additional permits might be necessary, negotiated and permitted by the administration of the National Park itself. Official and local permits should include the name of each field crew member on the permit, and the explicit permissions for entry of special geographic locations such as National Parks, restricted/prohibited areas, private land etc., and allowance of

collection of specific species (especially CITES, threatened, endangered or protected species) including any potential by-catch or the collection of other disciplines if applicable. Applications for these official and local permits should be made well in advance of the planned field work with adequate time for processing (and return) of the permits. Illegal fishing without permission may result in fines and other penalties (including prison time in some countries). Any permit conditions should be strictly adhered to (limitation of number of specimens of individual species, return of unwanted material to the environment, return of collected material to the country of origin after the collecting trip, restrictions on methods of collection, etc.).

2.2. Export / Import Permits

An export permit is required if the sampling/field work is carried out in a foreign country and the samples have to be transported from this country or state for further study and analysis (and/or final deposition). If the material is deemed for final deposition at the destination institution, the export permit should specifically allow the permanent export from the host country and transfer of ownership. The transfer of ownership may be negated by conditions such as export of CITES, endangered or protected species, or by other stipulations regarding the return of identified material to the host country. Again, as with collecting permits, any conditions or stipulations of the export permit must be adhered to. Import permits are required for the import of CITES species, but may also be required for the destination country and should also stipulate permanent deposition at the destination institution.

If the collected material is to be transported across federal/state, provincial or county boundaries, or exported and imported to different countries (e.g. for entry into the EU), national or international animal health and veterinary regulations may also apply requiring certificates for transportation, *i.e.* health or veterinary certificates, as detailed in Section 5 (Trade measures, importation/exportation procedures and health certification) of the *Aquatic Animal Health Code* as provided by the *Office International des Epizooties* (OIE). For further reading follow the OIE-link given below to Internet based information.

2.3. Additional permits and licences

Besides the above, further permits and licences may be required for any proposed fishing activities, including but not limited to:

- Valid electro-fishing licence at least for the collector who operates the dip net/the anode pole.
- Valid skipper's licence for the boat driver and/or coxswain (either a person of the fishing crew or a hired skipper) who knows the particular river very well (invaluable in navigating unknown, unchartered or dangerous waters).
- Diving certification for all persons who will be engaged in SCUBA sampling.
- Additional permission may also be needed for certain collecting methods, e.g. the use of rotenone (especially in freshwater environments), spear

guns, gill nets or any other method not routinely allowed by the general public.

2.4. Conservation and species protection regulations

Besides the relevant permits outlined above, the planned fieldwork has to comply with international wildlife regulations and provisions. The conservation status of the target species, and any potential by-catch in the specific collection area, needs to be evaluated before undertaking the collecting trip, to ensure that the necessary permits are in place prior to collection. Some species are protected or regulated by special legislation, such as CITES, the *International Union for Conservation of Nature* (IUCN), the *European Nature Conservation Legislation* or the *Endangered Species Act* (ESA), etc. Additionally, national or federal regulations on endangered species and species protection may apply or demand the obtaining of exemptions for catching certain species during closed seasons as well as undercutting minimum size limits.

2.5. Fish handling procedures and ethical concerns

Fishes are extremely sensitive animals that require fundamentally different handling requirements compared to other vertebrates due to their physiochemical make-up. Unnecessary by-catch together with careless handling and injury to specimens can result in increased mortality rates and must be avoided. Unnecessary (physiological) stress, inadequate handling or manipulation of specimens in the field will result in discoloured, damaged specimens with limited or no scientific value. Unless the fishes are not already dead (e.g. gill net fishing), fishes have to be euthanased prior to tissue sampling. An overdose of approved anaesthetic immobilizes the fish and allows more efficient processing and sampling of the catch and reduces potential pain at contact with the fixative.

Appropriate ichthyological anaesthetics include:

- Chlorobutanol (trichloro-2-methyl-2-propanol, CAS-No. 57-15-8); a saturated solution of approximately 1-2 tablespoons per litre will narcotise within seconds and has no known negative degrading effects on the DNA extracted from tissues. Possible issues:
 - a) For euthanasia, dip specimens 3-4 times for few seconds into the anaesthetic, but do not leave them for longer periods in the fluid; the adhering narcotic on the gill surface is sufficient for sedation.
 - b) Species with thick mucilaginous layers (e.g. eels, sturgeons) show increased mucus secretions after Chlorobutanol treatment caused by the high salt concentrations; especially those species should be narcotised by repeatedly short dipping for only few seconds.
 - c) Low ambient temperatures and metabolism rates of fishes during autumn/winter demand higher Chlorobutanol concentrations which may lead to vascular gill swellings and subsequent gill haemorrhage due to the high salt concentrations of the anaesthetic.

- d) Air breathing fishes are hardly affected and cannot be efficiently narcotised with this method.
- e) Chlorobutanol is extremely dangerous if ingested and may cause irritation of skin and eyes (see Appendix for further information and follow link for MSDS provided in section 7, internet based information).
- MS-222 (9-Tricaine Methane Sulfonate, CAS-No. 886-86-2) is the only approved substance (Europe, North America) for anesthesia of fishes; the fine powder can be dissolved in much lower concentrations (10 mg/l, thus avoiding possible negative effects of high salt concentrations of the narcotic; irritant but less irritating as Chlorobutanol (see Appendix for further information and follow link for MSDS provided in section 7, internet based information).
 - Clove oil (CAS-No. 8015-98-2) is a natural analgesic, the main ingredient Eugenol is used as narcotic mainly for marine organisms; Eugenol is water insoluble, for usage emulsify 1-5 ml clove oil in alcohol; irritant and hazardous in case of skin contact (see Appendix for further information and follow link for MSDS provided in section 7, internet based information).
 - Carbon dioxide can be an effective narcotic and is easily available e.g. carbonated bottled water or soda; narcosis can take longer with this method and may cause body contortions and muscle spasms that may affect the quality of the preserved specimens.

After short sedation in above detailed anaesthetics, or after electrofishing, fishes recover in well-oxygenated and ambient temperate water usually within minutes. For recovery, they should be placed in a separate tank or bucket. Only fully recovered specimens should be carefully released back into their environment to prevent injuries, damage or predation while still tranquilised.

2.6. Fishing relevant safety issues

Dangerous situations during fishing can arise within seconds leading to serious injury or fatalities.

Always ensure a firm, stable footing when collecting near or in water – especially when wading in fast flowing streams, in deep water or in case of low visibility of the water. Be especially cautious around slippery surfaces such as exposed, wet rocks, submerged substrates and vegetation or on moveable and uneven surfaces (sand, boulders etc.), and wear appropriate footwear such as rafting or canoeing shoes. Remember that waders can exacerbate such circumstances by causing extra drag and weight, especially when suddenly infiltrated by water. Attempt to strip off waders if in trouble.

When sampling with nets, beware of entanglement and drag caused by the net which can pull you off balance or into deeper water. If in trouble, release one or both ends of the net to maintain your footing and allow the net to drift with the current to minimize the contact surface and to reduce the velocity pressure and drag force of the net. In case of net fishing from a boat, avoid that the propeller

snags on static or grounded nets or catches the net during retrieving it into the boat. Human life is at all times more valuable than any sampling equipment!

When electrofishing, special precaution should be taken against electrocution by using rubber boots and gloves. Make sure that only certified/trained persons are allowed to operate the electrofishing device (mandatory in several countries). In the event of a member of the team falling into the water or otherwise coming into contact with electric current, cut immediately the electric circuit by removing either the anode pole or cathode rod from the water. Stop the engine before trying to help imperilled persons! Negligence of these simple principles will endanger further lives.

When diving or snorkelling, ensure that all safety precautions are taken and that one member of the team is always on shore to assist in an emergency.

A mobile or satellite phone and GPS device should always be part of sampling safety/emergency equipment to allow for rapid contact of and position location by emergency crews in the event of an accident.

2.7. Fluid fixation/preservation hazards

Exposure to aqueous solutions or fumes of formaldehyde should be avoided by always working in well-ventilated areas or fresh air and through the use of approved protective equipment. Formaldehyde is not only noxious but is also a known carcinogen. Minimise direct exposure as best as possible (e.g. only open the fixation container to add further specimens). Latex gloves provide no protection against formaldehyde, use Nitrile or Neoprene gloves for protection. Do not wear soft contact lenses which absorb formaldehyde vapours and will trap them against the eyes (Cohen *et al.*, 1979, cited in Simmons, 2002).

3. Fishing strategies (fishing gear, fishing methods)

Most fishing methods applied for ichthyological sampling catch unspecific, thus it is difficult (or impossible) to target individual species without some element of by-catch. This by-catch can however be reduced through the employment of appropriate, habitat specific sampling techniques, while the collection of multiple species may necessitate the use of multiple techniques at a given site. Fishing efficiency is affected by multiple factors (specifications and selectivity of the gear, seasonal variation, habitat conditions, fish size, etc.) which, in return, directly influence the collection efficiency of the field project. The fishing gear has to be selected in terms of operational efficiency and the availability of target species with regards to the sampling location. "Target species" is used here as a technical term and may include single species confined to specific habitats (e.g. specific pelagic fish species confined to open-water habitats which must be targeted with specific net gear), but can also include the complete fish fauna from a specific collection site (e.g. for taxonomical collections of fish faunas from previously unexplored rivers or lakes). Depending on what is defined as "target species" for the specific field trip or collection event, the fishing gear has to be selected. During the survey, it might be necessary to adjust the fishing methods to provide the required quality and quantity of the catch. A variety of

gear types or repeated fishing at the same spot might be necessary to ensure the widest possible range of fish species and life stages. Spawning and migratory behaviour of the target species, habitat preferences of different life stages of the same species and a basic knowledge of physical stream parameters, such as velocity, conductivity, stream size, water depth, water temperature, underground conditions (muddy/sandy/rock) are crucial for successfully fishing and sampling. Aspects that influence fishing efficiency are:

Water depth

- Deep water bodies such as lakes or large rivers favours long-line fishing, ground nets, fish weirs (if velocity allows).
- The length of the anode rod limits electrofishing (which normally ends 1 m below the water surface).

Water conductivity

- The salt concentrations in rivers and streams (depending on the geological conditions of the drainage area), at estuaries or entering freshwater streams in euryhaline lakes, in tidal pools and estuaries may vary extremely (*e.g.* favours or limits electrofishing).
- Different salt tolerances/preferences of euryhaline/stenohaline target species.

Water clarity

- Influences snorkelling/SCUBA diving.
- Electrofishing efficiency is strongly influenced by the clarity of the water.

Velocity

- Most static nets can only be deployed in shallow/stagnant water or in the direction of the current at moderate velocity.
- Higher water velocity drastically decreases electrofishing efficiency because stunned fishes will drift faster while increased drag on the anode pole will reduce its manoeuvrability.

Different behaviour/activity of target species

- Pelagic/demersal/benthic species.
- Diurnal/nocturnal species.
- Aestivation/hibernation.
- Spawning migrations.

Remark: Small species sheltering themselves in the shallow water from large nocturnal predators are easily caught at night in the shallow water using headlamps, handnets or a beach seine.

Habitat conditions

- Cobbles, boulders and rocks shelter lithophile or rheophile species.

- Benthic species may hide in muddy or sandy ground.
- Large stones, branches and trunks of trees minimize application of net gear.

Be aware that all of these parameters and conditions can change and normally do change within minutes during heavy rain events, e.g. in the rainy season. This not only strongly influences the fishing events following after the rain event because of increased turbidity or cloudiness of the water. Heavy rains (especially overnight) might also necessitate immediate removal of static fishing gear to avoid damage or loss.

Generally speaking, there are two different fishing methods – active and passive. For passive methods (such as gill nets or fish traps), personnel are only required for deployment and retrieving of the gear. Active methods require (with few exceptions) at least two people to actively operate the fishing gear during the collecting period. While active gear can be adapted during fishing e.g. for difficult habitat conditions, passive gear cannot. A combination of active and passive fishing methods will raise the sampling efficiency at a collecting site through setting of passive gear before the start of any active method and retrieving thereafter.

3.1. Selective (active) fishing methods

3.1.1. Cast (throw) net (Fig. 1A)

Operation

- Small net thrown onto the surface in a circular formation.
- After sinking to the ground the net is closed and retrieved.
- Requires trained skilled person to successfully use a cast net (for further information follow link provided at Internet based information below).

Specifications

- Small mesh sizes (usually 0.8, 1.0, 2.0 cm).
- Net should have bottom pockets to hold the catch.

Application

- Used in streams, rivers, lakes.
- Can be operated either wading in shallow water or from the boat.
- Allows for fairly directed and selective fishing.
- Can be used for retrieving live fish for bait.
- Only applicable at low or moderate water velocities (net will collapse in higher currents before reaching the ground).

Remarks

- The sample site should be free from obstacles like fallen trees, branches, roots, cobbles or boulders to allow closure of the net.
- Repeated casting at the same spot can scare off nearby fishes through the splashing of the net.

3.1.2. Seine (beach/pole seine) (Fig. 1A)

Operation

- Easily deployed from shore, wading in shallow water or from a boat.
- Requires minimal instruction and training.

Specifications

- Large variety of mesh sizes, lead lines and floats are available.
- Net specifications dependant on habitat and size of the target species.
- Should have a bunt (cod end) to effectively trap the catch.

Application

- Effective in most habitats but especially in larger streams, rivers and lakes.
- Shorelines should be free of obstacles to allow net to be pulled onto shore for effective specimen collection.
- Low to moderate water current.
- Beach seine with a fine mesh (up to 1.0 cm) should be shorter (approx. 10 m) to ensure that the seine can be pulled quickly enough against the current.
- Net filament can be either a strong visible (cotton) yarn or a less perceptible nylon or polyester, which provides lower visibility and detection by the catch.
- The lead line must be kept at ground level and ahead of the float line to prevent fish from escaping under the net.
- The float line must be raised well above the water surface when pulling the seine towards the shore to prevent fish from jumping over the net.
- Operating larger beach seines (20 m or more) requires a larger mesh size (to lower the velocity pressure) and more people to pull the net (because of increased drag force).
- Pole seines are usually operated from boats in deep water, or by wading in calm water (e.g. lakes).

Remarks

- Water depth and current can affect the efficiency of a seine net.

- If the water conditions allow, nets should be set or operated from row boats (to prevent any propellers from damaging or curling up the net) or manually by multiple people.
- This method has the advantage of being operated quietly, reducing the possibility of scaring fish from the sampling area.

3.1.3. Frame net (Fig. 1B)

Operation

- Individual fishing in shallow water.
- Along beaches and under overhanging shoreline vegetation (except for stands of dense weeds).
- For kick-net sampling in shallow riffles.

Specifications

- Either as solid aluminium or metal frame (60 x 40 cm), collapsible (two solid connectable parts) or foldable frame with a solid bar and two movable arms.
- Fine mesh (2.0-4.0 mm) to collect small species and fish fry.
- Approx. 30-40 cm deep net sack.

Application

- In shallow riffles, in creeks, smaller streams, slip-off slopes of larger rivers.
- Frame net is rammed into the soft bottom of the river vegetation and quickly lifted up.
- During sorting of the catch the frame must be kept well above the water surface while the net sack should remain dipped into the water.
- As a kick-net it can be either dragged by the current over larger boulders and rocks or scraped against the current, lifting smaller cobbles and stones and trapping smaller lithophilic fishes.

Remarks

- Favour aluminium frames in areas in which strong-electric fish occur (e.g. electric eels in South America or electric catfishes in Africa) due to the amplifying effects of the metal frame.
- Keep the net sack closed when crossing/wading through moderate to strong flowing water to reduce the drag force.

3.1.4. Angling

Operation

- Moderately selective method especially for clear water habitats.
- Species can be targeted through specific baiting.

- Fish size and species depends on hooks (size and form) and bait used.

Specifications

- Different types of fish hooks and monofilament lines, weights, baits, fishing rods and reels.

Application

- Can be employed from shore or boat.
- Highly biased method for fish size and species.
- Most effective if the angler has a specific knowledge of the habitat and habitat preferences of the target species.

3.1.5. Hand nets (Fig. 1B)

Operation

- Very selective method which allows also observation of fish activity, behaviour and habitat occupation (while snorkelling or SCUBA sampling).
- For collection of relatively small species in shallow water or from the surface of deeper water.
- Allows specific and selective sampling of individuals (e.g. breeding pairs, gobies and their symbiotic shrimps, etc.).
- Can also be used underwater for snorkelling or SCUBA collection.

Specifications

- Commercially available (aquarium) hand nets.
- Fine mesh size on varying size circular or square frame with wooden or metal handle.

Application

- Additional method for collecting specimens that may escape from seine nets.
- For underwater rotenone collections.
- For chasing specimens in very shallow pools and streams where larger seines are rendered ineffective.

3.1.6. Spear (Spear gun, Hawaiian sling or pole spear)

Operation

- Most selective collection method to target individual specimens.

Specifications

- Varying types, sizes, power and spear type (single or multi-barbed).
- Rubber band or air powered spear guns with barbed spear.
- Pole spear – long spear powered by rubber band (Fig. 1D).
- Hawaiian sling – smaller rubber band powered spear.
- Hand spear – thrown into shallow water or at species close to the surface of deeper water.

Application

- In any water body.
- Usually used underwater while snorkelling or SCUBA diving (although restricted or illegal in some countries).
- Requires sufficient underwater visibility.
- Can also be used from the surface on shore or from a boat.
- Hawaiian sling: operated and fired much like a slingshot; rubber bands attached to tube or block through which spear is drawn back and aimed.
- Pole spear: rubber band is held in the hand while pole is drawn back through the hand to produce tension in the rubber band; aimed through extending arm in front of face (Fig. 1D).
- Hand spear: varying length and barbs; effective in collecting flatfish in shallow water.

Remarks

- Spear collection is usually prohibited on SCUBA for the general public and specific permission may be necessary.
- Care should also be taken not to lose the spear and not to fire towards hard substrates or surfaces.
- Caution should be exhibited when using spears to ensure that they are not aimed towards other people or misfired during handling.

3.1.7. Electrofishing

Operation

- Common survey method to collect specimens as well as estimate abundances, density and species composition of fish populations.
- Uses electricity both to stun and attract the fish with the positive pole before capture (Galvanotaxis).
- Needs two persons for operation, one operating the dead man's switch and the electrofishing device, the other for catching and collecting the stunned fish.

Specifications

- Effectiveness depends heavily on water conductivity.
- Influenced by the size of the water body and riverbed conditions (soft or hard ground).
- Various models are available that generate varying strength of electric field.
- Pulsed or non-pulsed direct current (DC).
- Portable battery (Fig. 1B) or gas powered backpack models for small rivers and streams or large stationary models are available which are operated either from shore or a boat.
- The positive pole (anode) usually forms a ring at the end of a 2 m fibreglass or wooden pole and holds fine mesh net.
- The negative pole (cathode) comprises a braided copper cable which trails several meters behind the operator in the water.
- Pulse speed, voltage gradient and current influence and trigger Galvanotaxis and cause fish to turn into the electric field and to be attracted towards anode pole.
- Rubber gloves and rubber boots must be worn to isolate the operator and collector and to prevent electrocution.

Application

- Most useful in small creeks, streams, rivers and shallow (littoral) zones e.g. in lakes, in smaller rapids.
- Additional barrier nets can be placed downstream to collect the catch.
- Operator must be trained and in several countries a valid electrofishing licence is required.

Remarks

- Anode rods with dead man's switch included in the rod should be preferred instead of electrofishing devices with separate dead man switches operated by additional crew members (e.g. boat driver) for safety reasons, as the electrofisher operating the anode rod has the best overview in case of emergency.
- Stunned fish must be continuously removed from the electric field and be collected in plastic buckets or containers by additional fishing crew members to avoid injuring the fish through long term muscle contraction or tetanus.
- A continuous tetanus may easily break the backbone of smaller specimens.
- Fishing should be conducted in upstream direction so that disturbed debris and sediment are washed downstream and the visibility of the water remains good.

- Also minimises the escape effect of the cathode and allows for easy collection of the stunned fish that are swept downstream towards the collecting crew.
- The electric field depends not only on the conductivity of the water, but also on the ring size of the anode rod (see Table 1).
- Bedrock and sand bottom are insulating and support a stronger electric field in the free water column while soft or muddy bottoms are weakening and may even cause a collapse of the electric field (this might necessitate shortening the cathode length and thus the surface area with a cable strap).
- Wearing Polaroid glasses increases the visibility of both targeted fish and obstacles under water.
- Using a metal boat as cathode is strictly prohibited in several European countries for safety reasons.
- For further practical information please refer to Section 5.4.1 of the Fish Collection Methods and Standards Manual (website address see below at Internet based Information).

3.1.8. Fish market

To obtain a fast overview for the fish biodiversity especially in large rivers or lakes, it is always valuable to explore and obtain specimens from local fish markets. Local fishermen know the specific fishing grounds in their area well and normally apply a variety of different fishing methods which can provide a surprisingly high species richness. Depending on the climate and geographical region, these fish markets are either stocked early in the morning (before sunrise) or late in the afternoon. It is best to visit fish markets during these times to ensure freshness. Specimens should be sampled immediately on the market or might be carried cooled (e.g. placed on ice if available, but might be problematic in remote areas) possible for later sampling to ensure specimen and tissue viability. Fresh fishes are easily recognised by their transparent fins and vividly red coloured gills when lifting the gill cover. In most cases these specimens will need to be purchased and should be purchased to be fixed as voucher specimens, but in some cases either fishermen are not willing to sell their catch (e.g. fishermen depending on subsistence fishing in remote villages) or a specimen of a rare species might be too large to fit in any preservation container. In cases like this, ask for allowance of tissue sub-sampling of gill filaments or fins and photograph the specimen, so that the images can be taken to serve as vouchers. Fresh and freshly smoked and dried (not fried) specimens obtained from a fish market are even suitable for DNA sampling. As much authentic collecting information as possible should be obtained from the fishermen (fishing location, habitat conditions, fishing gear, fishing time, etc.), keeping in mind that might be imprecise or wrong, as some fishermen are not willing to reveal exact locations or fishing methods. Be aware that on large fish markets in major cities or capitals valuable species or complete catches might be carried by truck over hundreds of kilometres, stacked in layers of ice and sawdust to be sold for higher revenues. Sawdust in the fish baskets or on the

market place and bent or deformed fish bodies because of being stacked for hours or days are good indicators for this practise.

Commercial fishing methods are omitted here, since they are not applicable for small scale scientific surveys in freshwater environments. To expedite large lakes with trawling equipment, a commercial fishing crew and fish trawler should be hired.

3.2. Unselective (passive) methods

3.2.1. Gill net (Fig. 1C)

Operation

- Set across rivers and streams or on lakes (usually at night).
- Left in place for longer periods to allow specimens to become entrapped.
- Sampling areas should be free of underwater obstacles and have a moderate to low current.

Specifications

- Different types of net gear highly specific for species and fish size (standard gill nets, for targeting multiple fish sizes multi-mesh gill nets or enmeshing/trammel nets are used).
- Usually constructed of monofilament so as to be strong and invisible, but also as (visible) multifilament nets.
- Mesh sizes, floaters and weights might be optimised depending on the target species.
- Lead and float line should be sufficient to ensure net remains perpendicular to any current.
- Single nets can be combined to larger panels.

Application

- Highly effective when set in moderate to slow current in rivers, streams and lakes as a single net or as multiple sets in a staggered arrangement parallel to the shore, blocking preferred habitats or shelters (e.g. weeds or littoral zones).
- Can be effective for collection of species other nets will not catch (nocturnal, deeper water species).
- Net should be tied off to shore or a heavy weight to ensure net remains in place while floats should be large enough to ensure net does not get dragged under.
- For level inventories nets and panels employed at different depths (e.g. for target species with vertical or diurnal/nocturnal migrations).

Remarks

- Monofilament nylon nets should be cleaned after usage from any biofilm or algae coatings to maintain the invisibility.
- Areas with high fish abundances might require repeated control of the nets to minimize the number of killed fishes and possible negative effects on the fish population.
- Reducing the net size (single nets vs. nets combined to large panels) also helps to reduce the catch and avoids over-sampling.
- Might also be used as stop net for electrofishing or invisible seine in small streams with low risk of damages of floating debris (branches, leaves, etc.).
- For further practical information on gill nets and their application please refer to Section 5.3.2 of the Fish Collection Methods and Standards Manual (website address see below at Internet based Information).

Fig. 1 (next page). Active and passive fishing gear. A. Small beach seine (8 m) with lead line and floaters and approx. 1.20 m deep bunt; cast net with bottom pockets, diameter 8.0 m; B. Left, front: battery powered DEKA 3000 electrofishing device (out of production) with anode rod, collapsible frame net (net fixed with Velcro strip to aluminium frame); Middle: collapsible frame net, aluminium frame 60 x 40 cm, mesh size 2.0 mm, net sack 40 cm deep, frame included in special pocket in the net and secured with Velcro strip; Far right: Large dip net (approx. 1.0 x 1.2 m) with lead line and lateral short poles for manoeuvring the net; Front: staple dip nets (10 mm mesh, aluminium frame / 2 mm mesh, stainless steel frames, which might also be used as anode dip net for electrofishing), and hand nets; C. Monofilament gill net with lead line and floats (20 m, 10 mm mesh), fish trap with two traps and connecting wing (approx. 5 m long); Front: foldable fish traps without and with wings; D. Rubber band powered pole spear. (Photos D by M. Geiger, all other photos by D. Neumann).



3.2.2. Long-line fishing

Operation

- Short lines (up to 20 m) for sampling along shores or obstructive habitats like rapids or block stone embankments.
- Longer anchored lines (50 m and more) for sampling the river bed of large and deep rivers.
- Multiple hooks on branching lines and baiting may affect sampling efficiency.

Specifications

- Strong braided hook links with high strength fibres for maximum strength and abrasion resistance should be used.
- The length of the hook links should not exceed half the distance of the single hook links to each other to prevent the entangling of the hook lines.
- Small barbed hooks in combination with strong hook links allow catches of small to large species and minimize injuries and improves unhooking.
- Long-lines are usually anchored to the shore or substrate, additional weighting with lead is not necessary.

Application

- Long-line fishing is the only known method to sample the riverbed of large/deep rivers.
- Long lines for river bed sampling must be employed and anchored from a boat and marked with buoys.
- This method is even applicable in strong currents.
- Application of 20 m long-lines from the shore/river bank requires two persons (one holding the baited line which is anchored to the substrate, the other is throwing the anchored free end perpendicular to the beach into the river/stream).
- In strong currents or rapids, the free end fixed to a buoy may be released with the current, the depth of the long-line can be adjusted with length of the buoy line holding the free end.

Remark

- Secure the hooks immediately after unbaiting/unhooking within polystyrene foam to avoid entangling of hooks and lines.

3.2.3. Fish traps (Minnow traps/Fyke nets) (Figs 1C, 2)

Operation

- Small portable wire or net baskets.
- Traps with wings designed to guide especially small demersal and/or benthic species into the trap.
- Fyke nets are large hoop nets that act as funnels to trap swimming fish.

Specifications

- Either small foldable net or wire traps with internal funnels leading to a collection chamber.
- For small species, up to 60 cm length and 30 cm opening diameter with small mesh size.
- For large (demersal/nocturnal predatory) species, larger traps with 5-20 mm mesh size, 2 m or longer with opening diameters up to 75 cm.
- Larger traps are available commercially or from local fishermen but more difficult to handle and normally inappropriate for scientific sampling (these can be set by the collector or can be purchased from fishermen with existing nets – the benefit here being talking advantage of local knowledge and skill).
- Either with two wings (up to 3 m length) which are attached to one trap, or two traps which are attached to one wing.

Application

- Usually placed with or without bait in the shallow, calm or low current water near the shoreline.
- Assembled and placed before dusk and removed in the early morning.
- Traps and attached wings can be placed under overhanging vegetation, littoral zones and in front of weeds.
- Fyke nets may set at the intersection of smaller creeks to block the free passage.
- Traps should be monitored regularly for (nocturnal) predators.



Fig. 2. Wagenia fishing with large bamboo fish traps in the Congo rapids at Kisangani (Democratic Republic of Congo). (Photo by U. Schliewen).

3.2.4. Fishing using ichthyocide (Rotenone)

Operation

- Traditional fishing method employed from indigenous tribes e.g. in Africa and South America.
- Utilising the extract from roots of certain plant species (especially those belonging to the genus *Lonchocarpus* and *Derris* or *Tephrosia*, the latter used locally in many villages in the Democratic Republic of Congo).
- Pulverised roots are placed in the water to release the active ingredient.
- Depending on the affected area, needs 5-10 persons with dip nets to collect the fish.
- Rotenone fishing can be a useful alternative in inaccessible habitats, SCUBA collections in deeper water and for the collection of cryptic and hole dwelling, otherwise inaccessible species.

Specification

- Commercially used broad-spectrum insecticide, pesticide and piscicide.
- Available either as fine brown powder or emulsified liquid.

- Classified as IATA air dangerous good (toxic, Class 6.1).

Application

- Powdered Rotenone must be solved in water using an emulsifying agent (usually detergent).
- Liquid Rotenone can be applied directly but should be diluted 1:10 first.
- After mixing, Rotenone is easily spread into the environment using smaller containers like plastic bags, bottles or buckets.
- Affected fishes show suffocating symptoms and either turn gaspingly to the water surface or fall to the bottom.
- Fishes should be euthanased in an anaesthetic immediately after capture (prior to death) to avoid further pain and irreversible abduction of the lower jaws and opercles (results in poor quality of preserved specimens).

Toxicity

- Extremely toxic to insects and aquatic life including fish – easily absorbed through the gills or trachea.
- Interrupts the electron transport chain in the NADH complex in mitochondria of aquatic animals and insects.
- Has only minor and transient environmental side-effects.
- Low toxicity to humans or higher vertebrates.
- Poorly absorbed by the skin and gastrointestinal tract of mammals (see Appendix for further information and follow link for MSDS provided in section 7, internet based information).

Remarks

- Rotenone is the most effective tool available because only small quantities are necessary and well suited for small scale sampling of cryptic, hidden fishes or shoreline fish communities.
- Application area should be free of thicker mud deposits with sufficient water visibility to allow easy detection and effective collection of those specimens falling to the bottom (specimens are easily covered by disturbed mud and become soon invisible for collecting).
- Sampling locations with strong currents should be avoided or the habitat should have some mechanism of containing the spread of the rotenone.
- Spread of rotenone to adjacent areas should be prevented (areas of current flow affecting easily larger areas than required).
- In small streams or creeks, block affected area up- and downstream with gill nets prior to rotenone application to catch those specimens which flee from the rotenone or affected specimens that drift with the current.
- Great care should be taken in frequented areas of nearby villages.

- Rotenoning from shore, boat or SCUBA should be evaluated regarding efficient collecting of affected fishes from the environment.
- The environment should be evaluated with this in mind together with an estimation of the number of specimens that will be affected in order to minimize large scale effects on populations and the environment.

4. Sampling and fixation

This section covers only the collection and sampling techniques for freshwater fishes for scientific (zoological) purpose, *i.e.* tissue samples and fixing voucher specimens in the field for final deposition in Natural History Collections.

The handling time needed to set and retrieve the fishing gear, and subsequent time to sort, tissue sample and fix the specimens, is often underestimated. As a general rule, 1 to 2 minutes per specimen should be calculated for retrieval, tissue sampling and fixation. This adds up to about 6.5 hours for 200 samples processed. Experienced crews with highly efficient sampling workflows will need less time, untrained ones may need even more. Also, this time frame can be greatly increased by any ancillary sampling requirements such as measurement and photographing. Tissue viability and natural coloration will quickly fade. Both can be extended by keeping the fishes alive as long as possible or through cooling the freshly dead specimens with ice.

All of these factors should be taken into consideration when planning a sampling event as these may determine: a) the number of specimens that can realistically be handled (how many of them can be photographed and/or individualised and tissue sampled); b) the number of sites that can be sampled in a day. This is especially critical when collecting previously unexplored regions and faunas to ensure well-preserved, well-documented, straight specimens and individualised tissue samples. Poorly documented and/or preserved specimens resulting from overambitious sampling events are not only of limited scientific value but are a waste of time and money (Figs 4E,G and 5F).

4.1. Necessary equipment and chemicals

Table 1 gives a list of minimally required field equipment for the adequate sampling and fixation of fishes in the field. This list omits any recommendations for personal equipment (such as multi-tools, headlamps for night-fishing, tripod chairs, rain covers, etc.). Appropriate footwear for fishing is discussed above. This suggested list may need to be adapted relative to the planned sampling and depends on the duration of the trip, destination and mode of field transportation which may allow only a minimal subset of this gear.

As a general rule, all preservation and fixation chemicals (together with any other hazardous substances like Rotenone) should be kept strictly separated from each other as well as from other field gear (especially personal items) to prevent any risk of personal injury and also contamination of tissues samples by residual formalin or formalin vapours. Under humid climates, formalin vapours may condense during cooler nights inside the box containing the sampling gear and may adversely affect other gear and degrade tissue sample quality.

Dissection tools, documentation materials, DNA-vials, towels and additional plastic ware for DNA-vouchers should be packed in a durable (aluminium) container that will withstand the rigours of rough field conditions.

Additional gear may be necessary for specialised sampling routines such as electrofishing (protective boots and gloves) or for live fish maintenance during collection (tubs, oxygen supply) etc.

	Items/Gear type	Specifications	Remarks
Documentation	pre-numbered field lists or field book	water resistant laser or ink jet print	numbering coherent with fish-ID tags and vial numbers
	pre-numbered field tags	water and formalin-resistant	e.g. paper printed or numbered plastic tags
	paper or transparent paper (2-3 sheets)	should be acid free	for additional location data to be included to the preserved specimens
	2 graphite lead pencil or graphite monolith pencil	with graphite lead	industrial polymere lead pencils are made of coloured polymers not waterproof and rub off
	2 pigment ink pen	water & alcohol resistant pen	e.g. EDDING 1880 profipen, Securline MarkerII
	1 GPS device	positioning	should allow positioning to nearest 5 m and should receive satellite signals in forested areas
	1 camera	analog or digital	documentation of life colouration
	photo cuvette/photo aquarium	30 x 5 x 10 cm	documentation of life colouration
	(negative/slide) films or memory cards	storage medium	sufficient amount
Fishing gear (minimum) – non electrofishing	1 pole/beach seine	mesh size max. 10 mm	
	2 gill nets	monofilament nylon net, mesh size 10 mm	for small species
	2 gill/enmeshing nets	monofilament nylon net, mesh size 20-60 mm	for large species, mesh size depending on target species

	2 aquarium nets	20 x 10 cm	for juveniles and to sort the catch; 2 nets per person for snorkeling
	1-2 frame nets	60 x 40 cm with a 30-40 cm deep bunt	preferably collapsible
Fishing gear (optional) – non electrofishing	1 cast net	mesh size 6-8 mm, with bottom pockets, diameter 4-8 m	net diameter depending on the cast netting skills
	2-3 minnow traps or fyke nets	fine mesh, with funnels and collection chamber	trap should not exceed 30 cm diameter, wing not 3 m
	1-2 longlines	20 hooks/20 m line with small barbed hooks and strong hookings	application either from shore or boat (requires additional anchors & buoys)
	angling/hook & line	1 spool and several small barbed hooks	especially in third world countries for fishing kids
Electrofishing gear	electrofishing device	see 3.1	suited model size and generated power dependent on habitat conditions and water conductivity
	anode rod with electro-shock dip net	anode ring with dip net (mesh size 6-8 mm)	electric field depending on the ring size; large ring (30- 40 cm) = larger but more diffuse field; small ring (20 cm and less) = more concise field
	cathode cable	braided copper cable	standard length 2-3 m; length might be adjusted (see under 3.1)
	1-2 dip nets	firm net ring, diameter ca. 30 cm	1 dip net per person (except for the electrofisher and operator of the device)
	2-4 buckets	10-20 l	for collecting the catch during electrofishing
	and/or 1-2 tubs	80-90 l	for collecting and holding the catch during electrofishing

	Electro-shock low voltage gloves	size depending on the persons	natural rubber gloves for use on circuits of up to 1000 volts
	Wellington boots/hip waders	size depending on the persons	
Preservation gear	1 set of dissecting tools	1 fine scissors 1 re-usable scalpel 2-3 sharp blades 1 medium forceps (serrated tips, manipulation of formalin vouchers) 1 probe 1 box of needles	minimum equipment list; two separate sets of dissecting tools for manipulation of tissue and formalin samples are mandatory to prevent formalin contaminations of DNA tissues; larger fishing crews should operate with at least two dissecting kits
	4% aqueous buffered formalin solution	10-20 l; ca. 1:9 dilution of concentrated formalin (37%)	be sure the formalin is buffered, or add additional buffer to keep the pH stable (especially in hot climates); 1 l 37% formaldehyde in solution as back-up and for injection of large specimens
	1 syringe (with strong needles and spare needles)	50 ml	for formalin injection of large specimens
	anaesthetic	minimum quantity: 2 l in solution	carry sufficient additional crystalline anaesthetic as back-up
	2-3 towels		to remove adhesive fish slime from the hands after sorting the catch (contamination risk) and to padding the photo aquarium for transportation
	multi fold sanitary paper towels (1-2 pack)	recycling quality, as supplied in towel dispensers	paper towels in recycling quality remain stable if soaked with fixative solution (compared to toilet paper or kitchen towels)

	2 square plastic food containers	30 x 20 cm (as large as available)	minimum number; should be as leak proof as possible (rely on quality products) to prevent formalin leakage
	1 aquarium net	20 x 10 cm	to dip the fish in the anaesthetic
	5-10 PE plastic bottles	1-2 l	for direct fixation without prior pre-fixation of small specimens
	1 measuring jug	500-1000 ml	to pour the fixative into the fixation containers
	2 plastic kegs (optional)	20 l	round plastic kegs with red screw-on lids with air and watertight O-Ring rubber gaskets, UN-X approved for storage and further fixation of pre-fixed specimens
	cheese cloth/cotton cloth (optional)	sufficient	for enwrapping preserved specimens for transportation
	2 plastic kegs	60 l	as specified above, one for formalin, one for anaesthetic storage,
	2 plastic buckets (optional)	10-20 l	for transportation/sorting of the catch
	2 large plastic tubs (optional)	ca. 100 l	for holding of captured fish; required for storage during electrofishing
Tissue sampling gear	separate set of dissecting tools	separate blades for re-usable scalpel 1 fine scissors 1 fine forceps (with smooth tips)	manipulation of DNA-tissues
	2.0 ml self-standing microtubes	200-400	pre-numbered, prefer renowned high-quality brands
	1-2 storage boxes	100-200 place boxes	large boxes are easier to handle compared to small 81 place boxes
	toilet paper	1 coil	required for cleaning of scissors/forceps after each sampling

	centrifuge tubes	50-100, self-standing form	for preservation of small fishes as DNA-voucher
	96% distilled ethanol	1 l	as back-up and to fill the centrifuge tubes

Table 1. Field and preservation equipment.

4.2. Documentation – general aspects

The precise, accurate, detailed documentation of all associated data is of vital importance for any biological sampling. The more information is collected on the geographic, taxonomic and habitat characteristics of specimens, the more valuable these specimens become to the scientific community. Ideally, exact location should be pinpointed with the aid of a GPS unit. This may entail single point data, start and end points of a transect or corners of a rectangular area. If a GPS unit is not available or practical, precise written, descriptive locality information becomes that much more important.

At all times it is crucial that all associated data (geographic, taxonomic and habitat) be stored together with the samples to ensure correct interpretation and matching of this data to specimens. All field notes, labels and ancillary documentation should be taken on weather proof, alcohol and formaldehyde resistant paper and with good quality ink or pencil (see Table 1). It is helpful to draw a picture of the sampling location highlighting features of interest (vegetation, current direction, physical features, etc.), habitat specifications and exact sampling location(s), gear used and species collected. As collecting gear or sampling site might be very specific for species or sizes, the precise documentation of the gear types used at the respective habitats adds valuable biological and ecological information to the collected specimens. This allows conclusions on daytime and habitat preferences of species or different live stages of the species. A comprehensive list (but not be limited to) of information to be collected is included in the Appendix (Documentation of Collecting Event). Any semi-accurate information must be omitted, *e.g.* habitat or locality information for specimens purchased from the fish market (see comments in section 3.1, fish market), unless the exact location is known.

4.3. Landing, sorting and euthanizing the catch

Any catch must be landed carefully. This applies especially to landing net collected species where entangling may cause damage to the specimens, *e.g.* curling beach seines with the catch and debris and stones up the beach. Instead leave the net gear (and the fishes) in the shallow water (being sure to elevate the edges of the net to prevent escape) and retrieve the specimens as quick as possible. This may be unavoidable in the case of gill nets that are left in the water for extended periods. Fishes should be removed from entanglements as carefully as possible to ensure no further damage is done to the specimen. Specimens should always be removed head first to avoid fin damage or de-scaling. Care should be taken with spines as they can damage the net, the fish and the collector. Bony spines (and their serration) are valuable

diagnostic characters for many species. Be aware that some species have poison glands associated with bony spines which can cause painful injuries or numbing and the affected area may cause dizziness or even severe allergic shock.

Living specimens recovered from electrofishing or retrieved alive from fish traps or hooks might require temporary holding in large buckets or tubs for later euthanasing or photo documentation of live colouration. The water temperature should be ambient and the containers placed in the shade protected from direct sun. Covering the container reduces stress and prevents escaping. Overcrowding should be prevented and the water should be exchanged at regular intervals to reduce physiological stress. Additional (pure) oxygen-supply from oxygen-bottles may be mandatory (*e.g.* for holding during electrofishing in several European Countries).

Rapid sorting of the catch is crucial:

- to obtain an overview of the number of included species;
- to separate those specimens required for DNA sampling;
- to release or fix the remaining specimens.

Especially in hot climates, specimens die rapidly and this immediately starts autolysis of the guts which may compromise tissue removal, particularly in predatory and herbivorous species. Sort the catch according to species or genera required for sampling. Dead specimens are either set aside for later tissue-sampling or immediately fixed in formalin. Specimens should be placed in appropriately sized containers where the total ratio of animal tissue to fixative does not exceed 1:3, enabling the specimens inside to float. Small dead specimens (up to 10 cm total length) for formalin fixation are immediately collected head first either into 50 ml centrifuge tubes (Figs 3D & 4H) or PE-plastic bottles (1-2 l). Plastic containers (buckets, bottles, tubes, jars, etc.) should be constructed of UV resistant material to ensure they maintain their integrity. Insert a folded (to prevent rubbing and removal of lettering), water resistant label with sampling data written either with pigmented ink or pencil and fill the bottle to the top with the fixative solution (to prevent desiccation of specimens extremities). Leave the container horizontal for at least 1 hour to ensure that the specimens remain straight and do not bend during pre-fixation. After this pre-fixation, the container can be turned in upright position for thorough fixation of specimens (1-3 days, depending on specimen size, Figs 5D & 5F). Remaining specimens should be kept alive and fresh as long as possible to keep (and document) their living colouration. Dead specimens can also be placed on ice to maintain tissue integrity and colouration, especially if photographing or additional treatments are needed. Specimens may be euthanized immediately before tissue sampling and fixation by (repeated – if necessary) exposure to a suitable anaesthetic for few (5-10) seconds (see 2.4 for anaesthetics and doses). Sort the fish to be tissue sampled according to species as best as possible, since carrying forward the same species information into the tissue sampling lists is much more convenient and less time consuming. Euthanize only enough specimens as can be processed by the sampling crew in a single session. Depending on the species and fish size, if

opercular movements have ceased for ca. 5 minutes, the fish can be considered to be dead. Be aware that air breathing species, such as some Cyprinids, many Silurids, Gouramis and Lungfishes, or fishes with low respiration rates (e.g. during winter), are less perceptible for water soluble anaesthetics and may require repeated exposure to be euthanased. The live colouration should be documented (written description or photographed) prior to or shortly after euthanasing, but before fixation, since the pigment cells will relax and expand turning specimens dusky after treatment.

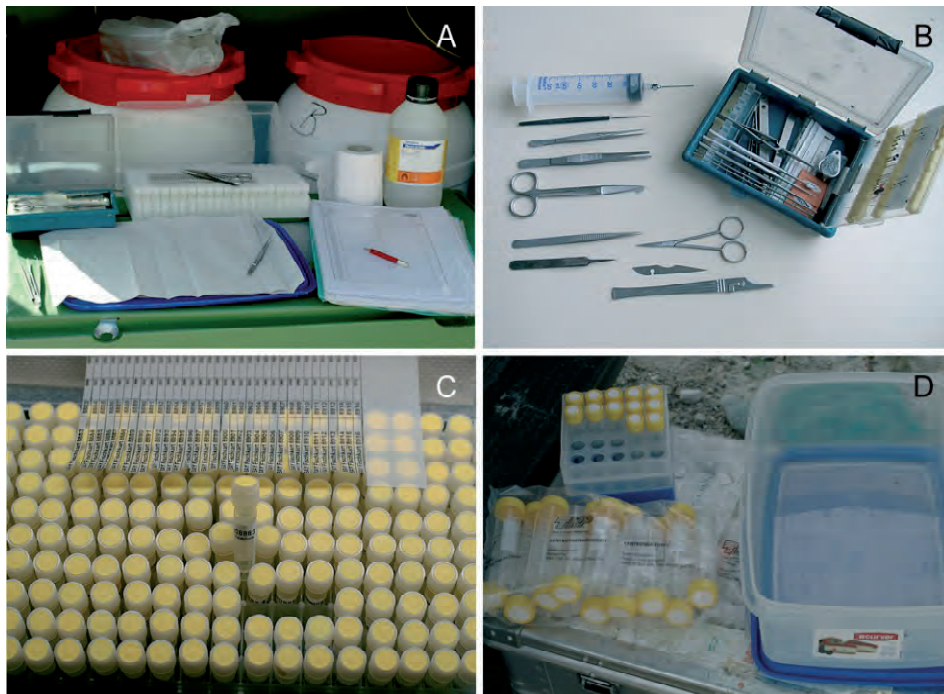


Fig. 3. Preservation and dissecting gear. A. Mobile tissue sampling station and documentation in a van: right, keg drumstores anaesthetic; left, 4% formaldehyde solution; euthanased specimens are sorted and tagged on fresh paper towels to minimise mucus contaminations, dissecting tools cleaned with absolute ethanol and toilet paper; B. Dissecting tools including two smooth forceps, scalpel blades and a small scissor for tissue sampling (bottom), different sized probes and needles for raising and fixing fins, larger serrated forceps for manipulating formalin specimens, large scissor and Luer Lock syringe for penetration of the abdominal cavity of larger specimens; C. Pre-numbered 2 ml storage vials (NUNC), 200 vials per rack, with corresponding gill tags (smooth tracing paper 110 g/m² printed with HP DeskJet 600 with original HP Cartridge no. 29); different sizes of the same numbers for tagging of smaller (first two numbers only) or larger (complete tag) specimens; D. Self-standing 15 ml and 50 ml centrifuge tubes (TPP) for fixation of small specimens and fixation container (commercially available plastic food container). (Photos A, B, D, F by S. Beyer, all other photos by D. Neumann).

4.4. Tagging of specimens and preservation of DNA-tissues

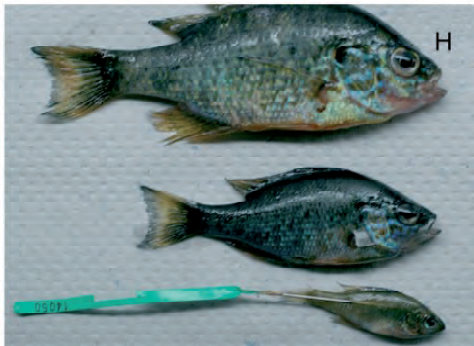
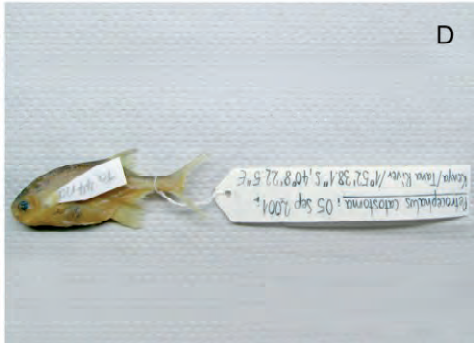
While formalin fixation varies only in relation to specimen size, tissue sampling necessitates exact and clean working to avoid cross-contamination of tissue samples, and should be done by a well-trained crew to speed up tissue sampling. Efficiently tagging and sampling of specimens requires two people, one for preparing, sorting and tagging, and the other for tissue sampling. At all times the link between specimen and tissue should be maintained both in field notes and through tagging of specimen and labelling of tissue tube. Tissue sampling requires a separate set of dissecting tools to avoid formalin contamination of tissues (for basic tissue sampling set up and requirements see Figs 3A-D). A set of freshly dead specimens (4-6, depending on size) are placed (sorted to species) with the head to the right on multi-fold sanitary paper towels to absorb residual mucus (Fig. 4A). Tissue tags can either be applied directly into the muscular tissue (Fig. 4C bottom, 4H bottom) or into the anus (Fig. 4C middle) using commercially available ribbon tags, t-end pins or similar (Fig. 4C), poly streamer tags (Fig. 4H, bottom) or tied through the gills (Figs 3C, 4B). Specimens are always tagged on the right side of the body, regardless which tag type is applied. In some species they may also be tied around the tail (Fig. 4D) ensuring that the tag will not work itself loose or become disassociated from the specimen. Tubes should be labelled with pencil or indelible ink, or both with information linking the specimen and tissue (Fig. 3C). Insertion of labels into tubes should be avoided to prevent contamination of tissue. The sampling crew should verify species and tissue tag numbers and tissue vial numbers during sampling to avoid mistakes during handling.

All tissues are sampled always from the right hand side of the specimen as the left hand side is traditionally used for measuring and photography. Muscle tissue is preferred to prevent having to gut the specimen for heart or liver tissue. Muscle tissue is usually removed from above the pectoral fin or on the caudal peduncle and should not alter the contour of the specimen (Fig. 4F). Muscular samples from the right abdominal region (behind the anus) are preferred if specimens have been dead for any length of time or if they have started decaying.

Fin clips are commonly sampled but may yield less DNA (quality and quantity). Usually only the lower portion of the pectoral fin is sampled, so that the total fin length remains unchanged and fin rays are still countable (both are diagnostic characters) (Fig. 4A, 4H top specimen). Depending on the specimens size, it may be necessary to cut the pectoral and pelvic fin, including the muscular fin bases (Fig. 4H, specimen in the middle), or to abduct the complete caudal peduncle (if more than one specimen is available) to receive enough tissue. The latter method should not be the first choice, because identification especially of small species under field conditions is extremely difficult, and recognising that the only minute specimen of a potentially new species lost its diagnostic character together with the caudal peduncle thereafter will neither improve the specimen, nor its condition. Cutting off bony spines has to be avoided for the same reasons, since most spines bear diagnostic characters and are essential for species identification as the left spine might be broken or missing (Fig. 4G).

It is best to use a new disposable scalpel blade for each tissue extraction, or to clean the scalpel blades or scissors after abducting the fins to prevent cross-contamination from one specimen to another. For this purpose, wipe and clean all tools (scissors, forceps, and scalpel) after each processed specimen best with 96% ethanol. If 96% ethanol is not easily accessible in the field, clean thoroughly with dry towel or toilet paper. While contamination between of allopatric species separated for a long period on a geologic time scale might be detected, it is impossible to detect cross-contaminations of just recently split or hybridising species. Same applies for any population genetics. The forceps for tissue manipulation should have smooth tips rather than serrated ones, to allow better cleaning of the tips and to avoid contaminations from residual mucus adhering to the serration. Excess mucus or debris should be removed from the tissue extraction site using paper towels or similar after which scales should be removed and the area cleansed (using 96% ethanol).

Fig. 4 (next page). Tissue sampling and tagging. A. Fin sampling of the lower portion of the right pectoral fin of euthanased sculpins; paper towels remove residual mucus; B. Gill tags should be folded with a small hook at the proximal end of the tag which is applied between 1st and 2nd gill arch (at least 2/3 of a gill tag should be covered – and secured – from the opercle); C. Application of different tags (from above): gill tag printed on smooth tracing paper with HP 600 DeskJet printer, T-anchor tags (Hall Print) applied into anus are firmly attached by piercing the gut canal (mind not to damage genital papillae during tagging to allow sexing of specimens thereafter), DYMO-tags applied with t-end pins and commercial tag guns (badly damaging especially small specimens); D. Small Mormyrid with handwritten tag (pigmented ink on durable paper) tied to the tail (museum gill tag easily lost and inappropriate for field conditions); E. Coiled up specimens removed from a museum jar: large t-end pins and even larger rigid plastic tags fixed to small specimens impede straight fixation and further damage them during museum storage; note that the imprint of DYMO-tags gets illegible in alcohol after few years; F. Muscular tissue samples should be removed from the tail (behind the anus) without altering the contour of the specimen. G. Diagnostic pectoral spine removed in the lower specimen for tissue sampling. H. Abduction of the fin base in smaller specimens might raise the DNA-content of the tissue sample; alternative PE-plastic streamer tag attached to a needle, applied in rostral direction into the muscular tissue causing minimal damage to the specimen (Caution: for museum storage plastic tags should be replaced because of potential corrosion from denaturing agents and/or high alcohol concentrations). (All photos by D. Neumann).



Tissues should be placed immediately into labelled tubes (preferably good quality, self standing, gasketed tubes), filled with 96% ethanol and tightly sealed (Fig. 3C). Cryo-tubes, which are especially designed for cryo-storage at deep temperatures, might cause problems under hot climates because of high evaporation losses. Repeated cases of evaporation losses of 50% or more have been reported. Be aware that in such cases the concentration of the residual ethanol may be far below 96%, since ethanol below 80% evaporates as pure ethanol. Tubes with 50% evaporation losses are inappropriate for tissue preservation and should be discarded since the ethanol concentration might have dropped below 40%. Prefer 96% distilled instead of chemically dried ethanol (concentrations of 99% or higher) as residual low boiling benzines used for drying the ethanol might degrade the DNA. The amount of tissue in any tube should not exceed one third of the total volume to allow for efficient and rapid preservation of tissues. If not avoidable, replenish with new ethanol after 2 hours. Tissue may also be cut into smaller pieces or macerated to facilitate this process.

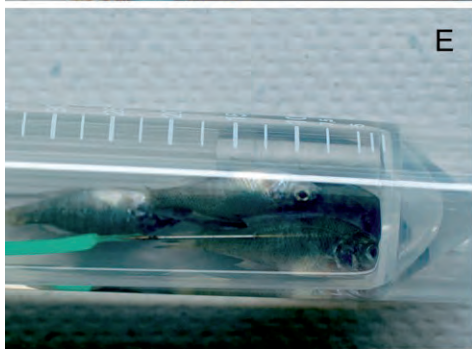
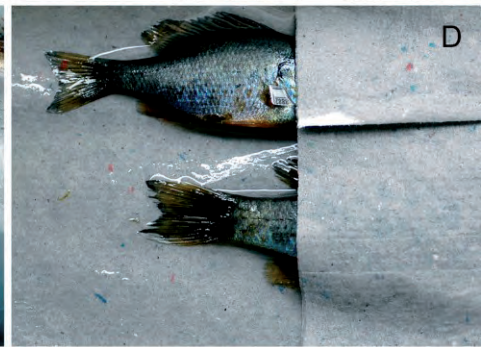
If no field tags are available for some reason, species should be sampled in order and by size to provide a mechanism of verification. For smaller specimens, whole specimens may be placed in tubes as tissue voucher but either photographs should be taken as vouchers or other specimens undoubtedly of the same species can serve as surrogate vouchers (not preferable).

4.5. Formalin fixation of specimens

Fixation and preservation is not the same. Preservation should only follow adequate fixation. Fixation stops autolysis by cross-linking and degrading proteins into amino acids by the formation of covalent bonds and coagulates cell contents to insoluble substances, whereas preservation alters the hydrogen bonding pattern and preserves the specimen by de-hydrating it (Simmons, 2002). Therefore, DNA-extraction of ethanol preserved tissues is possible (with DNA quality strongly depending on quick and efficient tissue dehydration), while extraction from formalin fixed tissues yields only short degraded gene fragments, depending on the number of cross-links and bonds which prevent the uncoiling of the DNA-Helix and thereby disabling the bonding of DNA polymerase.

The fixative should penetrate the specimens as rapidly as possible to prevent decomposition, especially of the guts and their contents. This initial or pre-fixation should be done within 10 minutes of the specimen dying.

Fig. 5 (next page). Fixation of fish. A. Prevent fusiform specimens from turning during prefixation; B. Prefixation of unpaired fins requires roughly 5 minutes; C. Orientate specimens in the one direction with sufficient spacing; D. Specimens stacked in different layers head on tails separated with formalin soaked paper towels; E. Small specimens (tissue sampled or ethanol vouchers) fixed head first horizontally in 50 ml centrifuge tubes; F. Nearly impossible to measure these bent specimens and useless for other morphological approaches; G. Opening abdominal cavity of large specimens allows quick fixation of the guts. (All photos by D. Neumann).



This 10-min time frame is crucial and requires a disciplined and experienced DNA-sampling crew and fixation routine.

The most common fixative is a 4% (or 3.7%) aqueous formaldehyde solution; in the tropics and for larger specimens, a higher concentration of 10% should be considered for quick pre-fixation (= 1:4 dilution of 37%, concentrated formaldehyde solution with tap or river water). Use only buffered, methanol stabilized formaldehyde solution (Simmons, 2002) for fixation to maintain a stable pH range as formaldehyde is unstable and oxidises in water into formic acid and to prevent decalcification especially of the often minute and fragile bones of fish skulls. While decalcification begins at a pH of 6.4 and below, clearing of soft tissues may already start at a pH of 7.0! Additionally, unbuffered formalin causes subsequent problems during later museum storage. Omitting the buffer from the formalin will shift the pH inside collection jars to acidic ranges. If you are dependent on locally available, unbuffered formalin because of transportation limitation (e.g. IATA aviation restrictions), this can be buffered through the addition of Sodium Phosphate Dibasic Anhydrous (CAS 7558-79-4) and Sodium Phosphate Monobasic Monohydrate (CAS 10049-21-5) in a ratio of 6 grams and 4 grams per litre respectively, or by adding a carbonate buffer; even chips of marble or limestone will help. Be aware that it might be necessary to add more than 6 (4) grams per litre of buffer, if you are depending on river water with low (acidic) natural pH values (e.g. streams draining rainforest or granite soils) for dilution of higher concentrated formalin to receive a 4% aqueous formaldehyde solution.

The diffusion rate of formaldehyde through the animal tissue is crucial and this may be slowed by thick or swollen mucus layers (e.g. in eels or sculpins) or size of the specimens. Simple formalin permeation (placing specimens directly into the fixative) is sufficient for fixation of small specimens up to 10 cm. Cover the bottom of the fixation container with 1-2 paper towels and a small amount of formalin, preventing the specimens from floating. This should allow the specimens to be fixed in a natural position. Better quality specimens may be obtained by raising the dorsal and pectoral fin with a probe thereby fixing the spines against the formalin soaked paper towel together with spreading the caudal fin. This improves counting of spines and soft rays of preserved specimens. If necessary, hold the raised spines in place with a probe or fix one of the first spines with a small needle. The minute muscles responsible for the fin movements are fixed within 5-10 minutes (Figs 5A-B). This may not be possible for all specimens due to time constraints.

Gill covers and branchiostegal apparatus should be in a normal position, the mouth should be slightly open, and the jaws should not be abducted. If a specimen has suffocated prior to relaxation in the anaesthetic, hold the fish and simultaneously keep the gill covers closed with one hand, while carefully closing the mouth and setting the branchiostegal apparatus back into a normal position with the other. Cut a finger off a disposable glove, push the specimen into the closed end and place it carefully into the fixative. Locked pectoral spines of catfishes should be released by pulling the spine carefully backwards (caudal direction) and turning it (beware of serrated or poisonous spines). All specimens which are placed in one layer into the fixative should be orientated in the same

direction. Take care that the new layer is sufficiently moist with formalin and place the next batch of specimens in the opposite direction (heads to tails) and raise the new fins by placing them on the bodies of the previous layer. The vertical distance between single specimens should be wide enough to unfold the caudal fins (Figs 5C-D).

This method of stacking the specimens in different layers of paper towels has several advantages:

- Pre-fixation and fixation can be done in the same container while limiting the necessary amount of formalin and minimizing the amount of formalin vapour.
- Specimens inside the layers will support each other resulting in straighter preserved specimens for natural history collections (compare Fig. 5F).
- Different sampling locations can be separated inside one fixation box (in this case pay attention to fold the outer ends of the towels upward to prevent that single specimens slip from their layer and mix with other locations).
- During transportation the arrangement in layers stabilises the specimens during fixation.
- The layers will also reduce movement of free formalin solution inside the box which stabilises the box during transportation and lowers the risk of potential spillage.

For specimens ranging from 10-20 cm, the permeation method is sufficient for pre-fixation only (10-20 minutes). After this time, the specimens need to be immersed in 4% formaldehyde solution to ensure high penetration rates (e.g. by stepwise filling the fixation box to the lowest layer with formalin).

For good fixation results and efficient penetration of specimens larger than 20 cm, the fixative may need to be injected into the body cavity. Assure that formalin/fixative is injected only into the cavity and not into the muscular tissue, which leads to intramuscular tissue rupture and formalin swelling of the tissues. Penetrate the abdomen laterally through of the anus or the belly near the (scaleless) base of the pelvic or pectoral fins with a syringe and needle (preferably a Luer Lock syringe and needle to prevent the needle from being propelled off the end of the syringe due to pressure build up inside the specimen's belly). Caution: To prevent eye damage through exposure to formaldehyde always wear protective eyewear (safety glasses or goggles) and turn the specimen away from your face when injecting and removing the needle.

Specimens larger than 30 cm should either be injected with 10-37% formaldehyde solution (37% for specimens larger than 50 cm) or the abdominal cavity should be opened with a sharp scalpel. Insert the scalpel into the anus (blade in parallel direction with the inner body cavity), cut 2-3 scale rows upwards in a dorsal direction and then turn in a rostral direction and parallel to the ventral border of the belly so that the natural contour of the specimen remains unchanged (Fig. 5G). Care should be taken to avoid damages of the genital papilla to ensure sexing of the specimen and to cut only the lateral right side of the body to keep the pre-anal measurements of this specimen.

Large specimens need to be placed in larger UN-approved, tight closing plastic kegs. For fixation it is important to leave the drums in a horizontal position for at least a day for good fixation results and to avoid deformation of specimens. For transportation, place the keg horizontally in a plastic box to collect any potential leakage and secure against rolling.

4.6. From fixation to preservation

Small to medium sized specimens should be left in sufficient formaldehyde solution in the fixation box for at least one week. Larger specimens should be checked periodically to ensure adequate fixation of the entire specimen – the belly should be firm, muscular tissue should be moderately hard, leaving no thumb imprint behind after manual inspection. If the gut contents of specimens (mainly herbivorous) start to decay, fermentation may cause gas to build in the gut cavity distending the specimen. In this case, the abdominal cavity needs to be opened and washed out thoroughly. Care should be taken when opening the abdominal cavity of such a specimen to prevent chemical eye burns and this can be done underwater to alleviate any pungent smell emanating from the specimen.

After fixation of specimens, the transfer to the preservation fluid has to be done in several steps to remove residual formalin from the specimens, and to avoid dehydration or cell rupture. First, wash the specimens by rinsing in water or by immersing in water and exchanging the water several times on a daily basis. This procedure should be repeated, until no or only a moderate formalin smell is perceptible. Then transfer the specimens into a 20% ethanol solution for 1-2 days (for specimens up to 5 cm), one week (specimens up to 20 cm) or 1-2 weeks (larger specimens), and repeat this procedure with 40% and 60% solutions before finally transferring the specimens into 70% ethanol. From personal observations, specimens originating from high water conductivity environments (especially European Cyprinids) seem to be highly susceptible to ethanol dehydration and thereby should not be transferred directly from formalin into 70% ethanol to avoid shrinkage.

5. Packing for transport

Returning samples from the field, including DNA-tissues as well as formalin preserved specimens might give rise to some unforeseen difficulties. Specimens should therefore always be packed in such a way that: a) they are not damaged during the transit; b) the specimen can be stored under stable conditions inside the containers for several weeks. This might be necessary if an airline refuses to carry formalin samples and you are forced to return the samples by surface mail from third world countries.

5.1. Transport on the road from the field site

Packing requirements of the specimens strongly depends on the road conditions.

5.1.1. On paved roads

Preservation and fishing gear has to be packed in such a way to prevent leakage from formalin containers and personal injury in case of emergency braking. All field gear should be secured in the trunk with straps, as for oxygen bottles and especially larger drums containing formalin or anaesthetics. Formalin containers should be placed in larger plastic or aluminium boxes to avoid any formalin leakage into the trunk particularly for bendy roads. Formalin and ethanol fixation gear always has to be stored in separate boxes to avoid any formalin contamination of the ethanol gear. Square plastic food containers are never 100% leak-proof and should be filled only to 5 cm below the top to minimise potential leakage. Formalin vapours emanating from the trunk into the cabin might cause dizziness or sickness to the driver. Plastic keg drums with formalin specimens should be carried horizontally or nearly horizontally (*e.g.* if a drum is placed and strapped into a tub) to avoid bending of only weakly fixed (larger) specimens inside the drum.

5.1.2. On gravel roads or rough tracks

For the transport of specimens for long distances and days over rough roads or dirt tracks, the packing of specimens requires additional precaution to avoid physical damage to them. Smaller PE-plastic bottles and kegs should be filled to the top with fixative leaving no air inside, to keep specimens in stable condition inside. Any free air space inside will shake both fluid and specimens vigorously after every road bump likely damaging the fins and abrading specimen-ID labels or DNA-tags. To protect fixed specimens in a keg or drum, wrap medium to large specimens in formalin soaked cloth. Each wrap should include only (consecutive series of DNA sampled) specimens from the same location to allow identification of specimens in case single field tags become illegible. Small fixed specimens can be easily separated and secured in disposable tea bags and several of those bags should be wrapped in cloth. For packing and returning material from foreign countries, decant the formalin and separate and pack the specimens in the same way. Additionally, single wraps inside the kegs may be packed inside aquarium bags and sealed tightly with rubber bands to minimise the risk of formalin leakage from the drums during transport. This method is not suited for pre-fixed or only weakly fixed specimens because the weaving pattern of the cloth (or specimens itself!) will imprint into the epidermis of the specimens!

5.2. Transport as carry-on luggage on board on aircraft

Carrying pure alcohol in hand or checked luggage onboard an aircraft is strictly prohibited (IATA, 2009). This applies for all DNA-samples *e.g.* placed in 2.0 ml tubes. Salt-based DNA-buffers (*e.g.* DMSO) or buffers with a volume less than 24% ethanol are not regulated. Alcohol based buffers should be referred to as "DNA-buffer" only when entering or leaving Islamic countries to avoid problems at customs. Aqueous formaldehyde solutions with less than 25% formaldehyde are not regulated under dangerous goods whereas solutions with more than 10% are classified as aviation regulated liquid (UN 3334). Concentrations less

than 10% are not regulated at all. To comply with the current IATA regulations, specimens should be wrapped in formalin (4%) soaked moist cheese-cloth, sealed in PE-plastic bags inside the drum as described above, and must be packed leak-proof in and best in UN-approved plastic kegs or drums. Note: There will be new regulations and amendments for shipping and transport of natural history specimens on board of aircraft in the 52st Ed. of the IATA Dangerous Goods Regulations affective on 1 January 2011 and perhaps in future; please keep yourself updated to comply with the regulations!

6. Acknowledgements

Most of the techniques outlined in this manual have been tested and adapted during the course of the project "Erfassung der Fischartenvielfalt Bayerns" (Species inventory of Bavarian freshwater fishes) 2003-2008 and various field work projects mainly in Africa conducted from ZSM staff (permanent and PhD students), sampling more than 10,000 individualised tissues and much more formalin specimens. The manuscript benefited from various experiences acquired during field sampling projects and/or input regarding field techniques from the following persons (alphabetical order): J. Freyhof (IGB, Berlin), M. Geiger (ZSM), T. Moritz, N. Pöllath, J. Schwarzer (ZSM), U. Schliewen (ZSM), E. Vreven (MRAC) and from various students deployed during the Bavarian species inventory. The manuscript gained from comments of two anonymous reviewers and especially from valuable contributions and critical comments from Andy Bentley (Ichthyology Collection Manager, University of Kansas) and Simon Moore (Senior Conservator of Natural Sciences, Hampshire County Council, Winchester). Without their help it would have been difficult to finish this manuscript under the given time constraints. Additional thanks for picture contributions to U. Schliewen (Fig. 2), M. Geiger (pictures in Fig. 1D) and Sebastian Beyer (Fig. 3A-B, D, F). All other photographs by D. Neumann.

7. References

IATA, 2009. Dangerous Goods Regulations, 50th Edition. International Air Transport Association, Montreal - Genf: 880 PP.

SIMMONS, J.E. 2002. Herpetological Collecting and Collections Management. *Society for the study of amphibians and reptiles, Herpetological Circular* 31: 1-153.

8. Further reading

For further information especially on net gear, electrofishing and fishing methods in general, the "Fish Collection Methods and Standards (Version 4.0)" is very valuable. This free Guideline issued by the Government of British Columbia (<http://ilmbwww.gov.bc.ca/risc/pubs/aquatic/fishcol/assets/fishml04.pdf>) can also be viewed (<http://ilmbwww.gov.bc.ca/risc/pubs/aquatic/fishcol/index.htm>) online.

9. Additional Internet based Information

CITES: <http://www.cites.org>

IUCN: <http://www.iucnredlist.org>

OIE: http://www.oie.int/eng/en_index.htm

How to throw a cast net: <http://www.ausfish.com.au/castnet/>

Aquatic Animal Health Code, Cpt. 5:

http://www.oie.int/eng/normes/fcode/en_sommaire.htm

10. Appendix 1 - Chemicals

Name	CAS-Number	Properties	Availability	Safety	IATA
Chlorobutanol	57-15-8	white, coarse crystalline powder	www.sciencelab.com VWR International	Irritant (eyes, skin) and extremely dangerous if ingested	not regulated
Ethanol (96%)	64-17-5	colourless liquid	www.sciencelab.com Sigma-Aldrich VWR International	Irritant (eyes, skin)	class 3
Eugenol	97-53-0	liquid oil		Irritant (skin), inhalation hazard; seek immediate medical attention in case of serious contact	may be regulated as UN 3334
Formaldehyde solution (10%)	5-00-0	colourless liquid	www.sciencelab.com Sigma-Aldrich VWR International	Irritant (skin), inhalation and ingestion hazard; carcinogenic, mutagenic and teratogenic effects (suspected or possible); classified developmental toxin to male/female reproductive system	concentrations of 10% and below may be regulated as UN 3334; concentrations of 10-24% regulated as UN 3334; less than 25% regulated as class 8 (UN 2209)
MS-222	886-86-2	white, fine crystalline powder	Sigma-Aldrich VWR International	Irritant (skin, eyes and respiratory system)	not regulated
Rotenone		brownish fine powder or dark	Sigma-Aldrich	Irritant (eyes and inhalation); after eye contact and ingestion seek	class 6.1

Disodium Hydrogen Phosphate	7558-79-4	clear or white crystalline hygroscopic powder	www.sciencelab.com VWR International	immediate medical attention; Lethal oral dose (Human): 143 mg/kg	not regulated
Sodium Dihydrogen Phosphate	10049-21-5	clear or white crystalline hygroscopic powder	www.sciencelab.com VWR International	Dust may cause irritation (eyes and respiratory system) Dust may cause irritation (eyes and respiratory system)	not regulated

11. Appendix 2 - Material Safety Data Sheets (MSDS)

Chlorobutanol: <http://www.sciencelab.com/xMSDS-Chlorobutanol-9923417>

Disodium hydrogenorthophosphate:

http://www.chemicalbook.com/ProductMSDSDetailCB1242667_EN.htm

Ethanol: <http://www.sciencelab.com/msds.php?msdsId=9923956>

Eugenol: <http://www.sciencelab.com/xMSDS-Eugenol-9924007>

Formaldehyde solution (10%):

<http://www.sciencelab.com/msds.php?msdsId=9924096>

MS-222:

[http://caligula.bcs.deakin.edu.au/bcs_admin/msds/msds_docs/Ethyl%203-aminobenzoate%20methanesulfonate%20salt%20\(Sigma%20A5040\).pdf](http://caligula.bcs.deakin.edu.au/bcs_admin/msds/msds_docs/Ethyl%203-aminobenzoate%20methanesulfonate%20salt%20(Sigma%20A5040).pdf)

Rotenone:

http://caligula.bcs.deakin.edu.au/bcs_admin/msds/msds_docs/Rotenone.pdf

Sodium Dihydrogen Phosphate:

<http://www.sciencelab.com/msds.php?msdsId=9925021>

12. Documentation of Collecting Event

Collecting Event

- Field number (usually provided by the collector) e.g. XYZ-2009/01 (three-letter country code-year/sample number).
- Date of collection – start and end date if necessary.
- Gear used – type or combination of different gear types.
- Gear specifications – mesh size, depth, exposure time, voltage/current (as precise and detailed as possible).
- Habitat specifications referred to employed gear type – (gill net in front of weeds, kick-net sampling in riffles, etc.).
- Habitat conditions referred to collecting site (hard/soft/sand bottom).
- Full names of all collectors (first, middle and last names).

Locality

- Drainage – might be hard to discern e.g. for small streams or swamps.
- Geopolitical designation (country, state/province, county, etc.).
- Detailed locality string – usually in 'named place' and 'location to named place' format.
- Highlight any distinctive features like road and river crossings (indicate any distances by road or linear – e.g. 5 miles West of...).

- Any other geographic subdivision (State, Province, Department, Village or Township, County (?) etc.).
- Latitude and longitude.
- Geodetic datum.

Specimen

- Identification to family, genus or species level (if known, otherwise unspecific as *e.g.* as “Cyprinidae indet.”, “Barbus sp.” to allow re-identification of tissues in cases of erroneous sampling lists).
- Any distinctive characteristics – colouration, morphological characters as these may be lost during fixation and preservation.
- Size – in millimetres as standard or total length (SL or TL) or as disc width for rays etc. (can slow down specimen processing and can be done at a later time).
- Weight – in grams (often times not practical in the field and may be done later).

Habitat

- Habitat specifications – road side ditch, floodplain, swamp, forest stream, rapids, etc.
- Peculiar habitat conditions – leaf litter, deadwood, dense weed standings, boulders, etc.
- Climate – cloud cover, precipitation etc.
- Season – rainy/dry season, summer/winter.
- Stream type – white water, clear water, black water.
- Water and ambient temperature.
- Water clarity.
- Water pH.
- Water conductivity.
- Current strength and direction.
- Associated vegetation.
- Associated species not collected.

Acknowledgements

No less than 79 authors from 14 different countries have contributed to the two parts that constitute volume 8 of the series *Abc Taxa*. Their names, institutional affiliation and contact information are put under each chapter heading, while their short professional biography can be found at the back of the second part of this volume.

In addition, thanks go to Marta De Biaggi (Torino), Merijn M. Bos (Arnhem), Pierre Commenville (Nice), Vanessa Demanoff (Paris), Henrik Enghoff (Copenhagen), Olivier Gargominy (Paris), Fabian Haas (Nairobi), Andreas Kunkel (Berlin), Marie-France Leccia (Nice), Benoît Lequette (Réunion), Bart Meganck (Tervuren), Patricia Mergen (Tervuren), Dominique Nandancé (Tervuren), Martin Nebel (Stuttgart), Monika Pfeffer (Stuttgart), Marian Ramos (Madrid), Patrizia Rossi (Valdieri), David Schindel (Washington, D.C.), Eduard Stloukal (Bratislava), and David Tarkhnishvili (Tbilisi) for their generous help and kind support granted in many different but essential ways for the project. Also, we would like to thank the very many colleagues, here unnamed, who contributed significantly to the completion of this volume by reviewing and commenting on individual parts and manuscripts.

A special word of thanks goes to Dr P. Bridgewater, Chairman of UK and international nature conservation advisory organisation JNCC (Joint Nature Conservation Committee), for providing the text on the back-cover.

The permanent (Y. Samyn, D. VandenSpiegel and J. Degreef) and *ad hoc* associated (J. Eymann, Ch. Häuser and C. Monje) editorial board of this volume of *Abc Taxa* would like to express its gratitude to all who have generously aided in the realisation of this book.

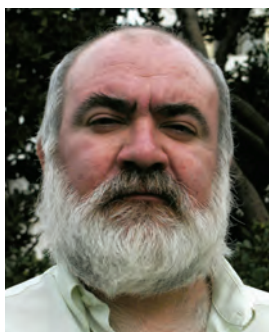
Funding for the production of this book came from the Belgian Directorate General for Development Cooperation through the Belgian National Focal Point to the Global Taxonomy Initiative and from the Sixth Framework Programme (FP6), Sub-Priority 1.1.6.3 "Global Change and Ecosystems" (project no. 018340), of the European Union through the European Distributed Institute of Taxonomy, partim WP7.



About the authors



Marina Aboal (°1959) is Full Professor and the head of the Phycology Research Group at Murcia University (Spain). She is interested in biodiversity and ecology of freshwater algae from the Mediterranean area. Although having studied lenitic and flowing systems in different regions she addressed in last years the algae communities and functioning of semiarid streams and the production of cyanotoxins by benthic communities. Her current research focuses on systematics of filamentous algae and its relationship with phosphorus dynamics.



Miguel Ángel Alonso-Zarazaga (°1956) is Tenured Scientist (Entomologist) at the Spanish National Museum of Natural History in Madrid (MNCN-CSIC). He carries out research on the nomenclature, systematics and evolution of Coleoptera Curculionoidea (weevils), particularly of the families Apionidae and Nanophyidae, and on the fauna of the Western Mediterranean and Macaronesia. He has described several new taxa of hypogean weevils and has more in study. Currently he has more than 130 published papers and, in collaboration with C.H.C. Lyal, runs a weevil database, WTaxa (<http://wtaxa.csic.es>).



Yves Basset (°1960) is scientific coordinator of the Tropical Canopy Biology Programme and Arthropod Initiative of the Center for Tropical Forest Science at the Smithsonian Tropical Research Institute in Panama. His main research interests are plant-insect interactions and canopy arthropods in tropical rainforests. During the past 25 years his professional residence was in Australia, Hawaii, Papua New Guinea, Guyana and Panama, with other field projects completed or extent in Gabon, Brasil and Thailand. Currently he is leading an ambitious programme to monitor arthropods in tropical forests.



Philippe Bouchet (°1953) is senior professor at the French National Muséum of Natural History, in Paris. He is a malacologist whose interests are with the exploration and description of biodiversity, especially marine invertebrates. Exploring for unknown faunas has taken him to expeditions in three oceans, as well as to a number of remote islands, with emphasis on the western Pacific, where he has coordinated several major international expeditions that have and have rallied the forces of as many as 80 scientists from 19 different countries working together to change our perspectives on marine megabiodiversity, attracting attention of the public, policy makers and scientists alike.



Peter Bridgewater (°1945) is currently Chair of the UK Joint Nature Conservation Committee and has been deeply involved in international biodiversity discussions for over 20 years. He helped organise the 'Darwin Conference' in 1998 which spurred the acceptance of the Global Taxonomy Initiative (GTI) by the Convention on Biological Diversity (CBD).



Bart Buyck (°1959) is associate professor and curator of the national mycological herbarium at the Muséum national d'histoire naturelle in Paris, France. He is also president of the French mycological society. His main interests are phylogeny and taxonomy of ectomycorrhizal basidiomycetes, particularly Russulales and Cantharellales. He has an extensive experience with fungal inventories from the tropics to the arctic zone. He participated in the Great Smoky Mts national park ATBI (USA), the ATBI in Santo (Vanuatu) organized by the Muséum, and he presently organizes for EDIT the fungal inventory for the ATBI in the Mercantour (France) - Alpi Maritime (Italy) national parks.



Santos Cirujano (°1950) is a researcher at the Real Jardín Botánico (CSIC) in Madrid. He is a specialist in Aquatic vascular plants.



Jan Decher (°1960) is a mammalogist by training and a research associate and lecturer in the Biology Department of the University of Vermont. His main research interests are the zoogeography, ecology, and conservation of West African small mammals. He has also worked as a small mammal consultant for the rapid assessment program (RAP) of Conservation international, for the environmental engineering firms NKUK and SNC Lavalin, in Côte d'Ivoire, Ghana, Guinea, and Sierra Leone, and recently for Heindel & Noyes, Inc. for an Environmental Impact Statement in southern Vermont.



Jérôme Degreef (°1966) is head of the department Cryptogamy and curator of the cryptogamic collections at the National Botanic Garden of Belgium, Meise. His research focuses on the taxonomy of tropical African fungi (mainly Boletales) and ethnomycology. During the past 10 years he has been involved in fieldwork all over Africa, collecting specimens for traditional morphology-based and molecular taxonomic research and compiling a checklist of edible mushrooms. He also is the co-founder and one of the permanent editors of the capacity building journal *Abc Taxa*.



Wouter Dekoninck (°1974) obtained his PhD in 2008 with a study on the distribution, ecology and conservation of ants in Belgium with emphasis on Flanders. Since 2000 he works at the Entomology Department of the Royal Belgian Institute for Natural Sciences in Brussels as scientific assistant, curator of databases and curator of the entomological collection. His main topics are sampling methodology, ecology and inventory of Formicidae, Carabidae and Culicidae; Red data analyses; Site Quality Assessment using invertebrates; knowledge on insect distribution, protection of threatened habitats/species. He is Belgian ant curator since 2006 and president of the Royal Belgian Entomological Society since 2009.



Jacques Delabie (°1957) is researcher at the Cocoa Research Center of CEPLAC, Brazilian Agriculture Ministry, and full Professor of Santa Cruz State University, Bahia, Brazil. His main research interests are ant biogeography, community ecology and diversity, as well as use of ants for biological control in cocoa plantations or biomonitoring. He has collaborated to several large research programs for ant taxonomy and ecology. He is currently participating to arborous and leaf-litter ant community and ant-plant interaction studies, firstly, but not exclusively, in the Neotropical Region.



Teresa Domingo-Quero (°1976) is a predoctoral fellow in the Spanish National Museum of Natural History in Madrid (MNCN-CSIC). She is currently finishing her PhD thesis on the Coleoptera diversity of the Caldera de Taburiente National Park (La Palma I., Canary Islands, Spain). In 1999 she graduated in Biology in the Alcalá University (Madrid, Spain). From 1999 to 2001, she worked in the ATBI project: 'The Invertebrate Fauna Inventory of Caldera de Taburiente National Park'. During two years of field work, she collected invertebrate samples using multiple techniques and processed them in the laboratory: soil, litter, vegetation, freshwater and caves were included in this study.



Gabriele Droege (°1978) is a computer scientist at the Botanic Garden and Botanical Museum Berlin-Dahlem. She studied Biology at the Freie Universität Berlin. After her Diploma thesis about rooks (*Corvus frugilegus*) she developed the Corvids Literature Database. Within the DFGfunded DNA Bank Network she is currently responsible for data architecture and software development. Her main interest is directed towards aggregation of biological data, especially all kinds of biological collections and publications.



Xavier Eekhout (°1977) is a herpetologist currently working in project management in the Museo Nacional de Ciencias Naturales (MNCN). Although he has mainly worked with amphibians, he also has strong personal interests in reptiles. Professionally he has studied frog population dynamics with the aid of drift fences, and later acoustic communication in anurans of Spain and Mexico. He has also participated as instructor in general bioacoustics courses in Mexico and regularly collaborates with the Fonoteca Zoológica (www.FonoZoo.com), the animal sound collection of the MNCN.



Jutta Eymann (°1972) has been working as scientific coordinator for the workpackage “Applying Taxonomy to Conservation” within the EU-project EDIT (European Distributed Institute of Taxonomy) while co-editing this Field Manual. Currently, she is back in Australia where she did her PhD on conservation and management of urban brushtail possum populations.



Jon Fjeldså (°1942) is curator of birds at the Zoological Museum, University of Copenhagen, since 1971, and is now also professor in biodiversity studies. Has long experience in expedition work in tropical countries, especially in the Andes of South America and East Africa.



Andreas Floren (°1961) is since 18 years involved in canopy research focussing on the diversity, structure and dynamics of tree specific arboreal arthropod communities, which are sampled mainly by highly efficient canopy fogging. Starting in SE-Asian primary rain forests he extended his research to anthropogenically disturbed forests. For about 12 years he is carrying out comparable studies in temperate Europe. There is no lack of managed forests in Germany, but for primary forests he had to change to the Polish Białowieża forest. These data form the basis for his recent research project focussing on the functional importance of xylobiontic arthropods (mainly beetles) by manipulating key resources like canopy and ground dead wood.



Antonio Garcia-Valdecasas (°1952) is a researcher at the Museo Nacional Ciencias Naturales (CSIC) in Madrid. He is a specialist in Hydrachnidia and has worked on taxonomic tools by computer. Presently he is working on the scientific foundations of taxonomic work.



Birgit Gemeinholzer (°1968) is scientific head of the molecular laboratory at the Berlin Botanic Garden and Botanical Museum (Freie Universität Berlin). She is heading the German DNA Bank Network and is involved in several projects to safeguard primary data of biodiversity research. Her own research interest is within the plant group Cichorieae/Asteraceae analyzing evolutionary and phylogenetic mechanisms but also covering topics on species delimitation and detection, like DNA barcoding.



Adriaan Gittenberger (°1976) is associate researcher at the National Museum of Natural History Naturalis, and the Institute of Biology, Leiden University (The Netherlands). He joins the marine expeditions of Naturalis as specialist of ascidians and gastropod parasites of corals. In addition, he directs GiMaRIS (cf. www.gimaris.com). Together with his team of biology and bioinformatics students he runs a multidisciplinary research program in temperate areas, focusing on marine species communities and population dynamics based on long-term monitoring, field studies and aquarium experiments and molecular analyses.



Robbert Gradstein (°1943) is a retired Professor of Botany, and former Director of the Herbarium and Botanical Garden of the University of Göttingen. His main research interests are systematics and biogeography of bryophytes, especially liverworts, and flora and vegetation of the tropics. Professor Gradstein had done fieldwork in all parts of tropical America and in Asia, has directed numerous research projects in his field of interest, and has published more than 350 research papers, including several books. He is co-author of "Guide to the Bryophytes of Tropical America" (2001), the first synthesis of its kind. Professor Gradstein is the editor-in-chief of the journal TAXON, member of the Academy of Sciences of Göttingen, and a former president of the International Association of Bryologists. For his botanical research he was awarded the Prix Augustin-Pyramus de Candolle of the Societé de Physique et d'Histoire Naturelle de Genève.



Patrick Grootaert (°1952) promoted with a PhD on freeliving nematodes at the University of Ghent, but later specialised in Diptera, Empidoidea when he became employed at the Royal Belgian Institute of Natural Sciences in Brussels in 1980. In the beginning he worked on taxonomy and faunistics of Palaearctic fauna. He helped to develop Red Data Books and performed many site quality assessments of forests, heath land and coastal dunes using various insect groups as indicators. He directs the Department of Entomology at RBINS since 1991 and his main interests are now in the fauna of Southeast Asia and in particular in mangrove fauna.



Michael Grundmann (°1961) is curator for molecular collections at the Botany department, Natural History Museum, London. His doctoral thesis, prepared at Heidelberg University, Germany and the NHM, was on phylogeny, population genetics and phylogeography of the moss genus *Pleurochaete*. During the past 10 years he has been involved in fieldwork all over Europe, collecting specimens for molecular research on bryophytes, ferns and flowering plants. His current work focuses on conservation and storage procedures of DNA and tissue collections.



Christoph L. Häuser (°1959) is a biologist with a background in taxonomy and evolutionary biology, originally specializing in the study of Lepidoptera. Since 2009 he is leading the office for international collaboration and science policy at the Directorate of the Berlin Museum für Naturkunde, and has been actively involved in several international efforts linking taxonomy, information science, and biodiversity conservation (CBD: GTI, GBIF). For the EDIT project, he has been leading Work Package 7 (Applying Taxonomy to Conservation), developing an ATBI+M approach for protected areas. He has ample field experience as an entomologist working recently at ATBI+M pilot sites in Europe, and also before in many countries in Asia, Africa, and Latin America.



Anke Hoffmann (°1962) is scientific coordinator for the workpackage “Applying Taxonomy to Conservation” within the EU-project EDIT (European Distributed Institute of Taxonomy) and is based at the Museum für Naturkunde in Berlin. Her PhD (1999) was on population dynamics and habitat utilisation of terrestrial small mammals in the grasslands of Queen Elizabeth National Park in Uganda. Moreover she has been involved in further small mammal surveys in different habitats, like in rain forests in Uganda and Guinea, also in the Nama-Karoo in Namibia. Currently she focuses on small mammal inventories in the EDIT ATBI+M sites of Slovakia (Gemera Area) and France/Italy (Mercantour/Alpi Maritime National Park).



Joachim Holstein (°1965) is research entomologist at the State Museum of Natural History Stuttgart, Germany. He is interested in afrotropical Saltatoria and Araneae, mainly Jumping Spiders (Salticidae) but is also very familiar with the insect fauna of Central Europe. His wide-ranging interest in entomological subjects as well as in IT technics results in several projects of designing database management systems for collection management, faunistics, and research. His current research focuses on East African jumping spiders and the insect fauna of Kakamega Forest in Western Kenya as well as on the development of an illustrated database and information system for the European fauna.



Valerie Hofstetter (°1964) is responsible for the molecular diagnostics of fungi at the Agroscope Changins-Wädenswil, Nyon, Switzerland. She is a molecular biologist interested in systematics of various groups of asco- and basidiomycetes. She participated in fungal inventories on many continents, where she had the responsibility for the barcoding aspects and tissue sampling for all fungal groups. She presently works on fungal communities of vine and other agricultural crops.



Sanda Iepure (°1974) is a Researcher at Speleological Institute “Emil Racovitza” Biospeleology Department from Cluj, Romania. She carries out systematic, ecologic, biogeographic and conservation researches on groundwater crustaceans (cyclopoids and ostracods) mainly from caves. Currently her researches are focused on speciation and phylogeny of cave cyclopoids and ostracods from the peri-Mediterranean region inferred from both molecular and morphologic approaches.



Damià Jaume (°1963) is CSIC staff Scientist at the Mediterranean Institute of Advanced Studies (Balearic Islands, Spain). His research is focused on the taxonomy, comparative morphology, biogeography and phylogeography of peracarid crustaceans, especially those living in anchialine environments and other subterranean habitats.



Lars Krogmann (°1976) is a research scientist and curator of Hymenoptera in the Entomological Department of the State Museum of Natural History in Stuttgart. His research focuses on the systematics and evolutionary biology of parasitoid wasps. He obtained a PhD from the University of Hamburg and has previously worked as a Feodor Lynen Fellow of the Alexander von Humboldt-foundation at the University of Adelaide in South Australia.



Alexander Kroupa (°1966) is graduated in Zoology focussing on community ecology and systematics at the University of Bayreuth and specialised on aculeate Hymenoptera (www.HymIS.eu). Subsequently working for several companies and consultancies developing IT applications for various biodiversity monitoring and conservation programs. Currently employed at Museum für Naturkunde in Berlin working for EDIT to test and improve field-based digital recording tools and techniques for biodiversity inventorying and monitoring programs.



Thomas Læssøe (°1958) is associate professor at the University of Copenhagen (Denmark) with an extensive field experience on many continents and in many climatic zones, from far Eastern Russia to lowland Ecuador. His main interests are with the taxonomy of ascomycetes, in particular pyrenomycetes (especially tropical Xylariaceae), but also has extensive experience with many saprotrophic basidiomycetes and several other fungal groups. He coauthors one of the most comprehensive, user friendly, multientry, computerised keys to fungi on and off the web (www.mycokey.com), maintains a webpage and database on Ecuadorean fungi, and co-runs the newly started atlas project in Denmark (www.svampeatlas.dk).



Gaël Lancelot (°1981) is the communication officer for the European Distributed Institute of Taxonomy, working at the Muséum National d'Histoire Naturelle in Paris. He has previously worked for the Global Biodiversity Information Facility and the BioCASE project. He is the author of *University Reorganisations in Biology: remodelling British and French life sciences, 1960-1995*.



Maurice Leponce (°1964) is researcher at the Biological Evaluation section of the Royal Belgian Institute of Natural Science. His main research interests are ant and termite community ecology and biodiversity. He has collaborated to large-scale biodiversity inventories in Panama, Australia, Vanuatu and France in the framework of IBISCA (Investigating the Biodiversity of Soil and Canopy Arthropods) projects. He currently studies the response of ant communities to environmental changes in Podocarpus National Park (EDIT's ATBI site in Ecuador).



Diego Llusia (°1978) is a PhD researcher in the National Museum of Natural Sciences (MNCN-CSIC) at Madrid (Spain). His research includes acoustic communication, behaviour and ecology of terrestrial vertebrates. He is currently involved in acoustic monitoring of anuran populations in light of climate change. He previously taught biology in SEK-Segovia University and was member of the Ecology of Steppe Birds Research Group in the Autonomous University of Madrid.



Raphael Marquez (°1962) is Tenured Researcher (Investigador Científico) of the Spanish National Research Council (CSIC). He directs the laboratory of Bioacoustics and the Animal Sound Library Collection of the National Museum of Natural Sciences in Madrid, Spain. His current research involves the development of acoustic monitoring programs in terrestrial habitats (anurans and birds).



Christopher Meyer (°1966) is a research zoologist at the Smithsonian National Museum of Natural History in Washington D.C. His main research interests are in diversification processes and patterns in marine systems, especially those associated with coral reefs. Generally, he uses diverse molluscan groups to test phylogenetic and phylogeographic hypotheses. His other research interests include the use of genetic markers to examine diversity in marine communities. Currently he is the director of the Moorea Biocode Project, an all taxon, voucher-based, genetic inventory of a tropical ecosystem in French Polynesia.



Marianne Meyer (°1943) is living in the French Alps. Notwithstanding the fact that she is an 'amateur' mycologist, she is one of the world experts on myxomycetes maintaining an intensive international collaboration and activity. She is particularly known for her contributions on nivicolous myxomycetes and is presently finishing a prestigious and beautifully illustrated world monograph. Her incredible energy and contagious enthusiasm for myxomycetes has made her one of Europe's best known ambassadors of this group.



Juan Carlos Monje (°1963) is a scientist at the State Museum for Natural History, Stuttgart, Germany. His expertise is in the classical and molecular taxonomy of minute parasitic wasps. He works at present as project coordinator and is one of the team leaders for EDIT's Work Package 7.



Alexandra N. Muellner (°1974) is a postdoctoral lab manager and research group leader at the Grunelius-Moellgaard Laboratory at the Senckenberg Research Institute in Frankfurt, and teaches at Goethe University. She set the stage for Senckenberg's DNA Bank and DNA database which cover various groups of organisms (plants, fungi, animals). Her research focus during the past 10 years has been on molecular phylogenetics, molecular phylogeography and historical biogeography of temperate and tropical flowering plants. Recently, research has centered largely upon taxa of the pantropical family Meliaceae (mahogany family) in the order Sapindales. Her scientific research resulted in various papers, book chapters (incl. floras), reports and media involvement. She has received several prizes and awards for her work.



Dirk Neumann (°1972) is diplom biologist and technician managing the ichthyological collection and in charge for the technical equipment in the DNA Lab of ZSM. Main interests are the ichthyofaunal diversity of Bavarian rivers and nilo-sudanian rivers in Northwest Africa. Besides coordination in the species inventory of Bavarian freshwater fishes, he was engaged in 8 field projects in South America and Africa the past 10 years.



Martin Obrist (°1958) is research associate in the Biodiversity and Conservation Biology research unit of the Swiss Federal Research Institute WSL in Birmensdorf, Switzerland. He is engaged in the development of biodiversity assessment tools in general as well as acoustic tools for the assessment of bat species by their echolocation calls in specific. Present work involves the acoustics part of the revision of the Red List of Swiss bats and a study on bats' response to habitat connectivity, which both will be analysed in the framework of spatial ecology.



Beáta Papp (°1965) is the curator of the Bryophyte Herbarium (BP) of the Hungarian Natural History Museum since 1999 she is also the editor of *Studia Botanica Hungarica*. She participated in numerous monitoring and conservation programs, in her home country Hungary but also in the Balkan, Turkey and in East Asia. In 2005 she received the Pro Natura Award from the Ministry of Environment for her efforts in the field of conservation of bryophytes.



Gustav Paulay (°1957) is curator of marine invertebrates at the Florida Museum of Natural History. He uses integrative taxonomy, large-scale biodiversity surveys and sequencing efforts to document and understand the diversity, distribution, and evolution of reef-associated marine invertebrates. His focus has been largely on tropical Indo-Pacific corals, molluscs, crustaceans, and echinoderms.



Gianni Pavan (°1960) is Professor of “Terrestrial and Marine Bioacoustics” at the University of Pavia, Italy. He is President of the “Centro Interdisciplinare di Bioacustica e Ricerche Ambientali” he contributed to create in 1989 to develop advanced bioacoustic research based on digital techniques. He started to work on computational bioacoustics in 1980. His main research interests are now on marine mammals acoustics and terrestrial landscapes. Cooperates with ONR, NATO, WHOI, IT Navy and other institutions worldwide to study marine mammals. He developed the SeaPro package for real-time sound analysis and display.



Gitte Petersen (°1963) is professor in molecular systematics of higher plants at the Natural History Museum of Denmark. Her research is focused on phylogens and molecular evolution of the monocotyledons.



Mark Pollet (°1961) was trained as an entomologist at the University of Ghent (1983) where he also accomplished his doctoral research on the feeding ecology of carabid beetles (Coleoptera, Carabidae) in pasture ecosystems. Since 1985 he grew a passion for scientific research on long-legged flies (Diptera, Dolichopodidae) from the Palaearctic and the New World, in all their aspects. As grant coordinator – scientific advisor sr at the Institute for the Promotion of Innovation through Science and Technology (IWT-Vlaanderen), between 1994 and 2009 he organized hundreds of evaluation committees and evaluated a multifold of PhD applicants in biology. Since 2009, he's head of the new Information and Data Center at the Research Institute for Nature and Forest (INBO), which combines his interest for nature conservation and information and data systems.



Heather Proctor (°1964) is a professor in Biological Sciences at the University of Alberta, Canada. Her early research was focused on the ecology and evolution of water mites (Hydrachnidia) but has since branched out to include the community ecology of freshwater and terrestrial invertebrates and the ecology and systematics of bird-associated mites.



David Rawson (°1943) is professor of Applied Cell Biology in the LIRANS Institute of Research in the Applied Natural Sciences at the University of Bedfordshire (United Kingdom) and Visiting Professor at the China Agricultural University, Beijing (China). His main area of research is cryobiology and the cryopreservation of cells, gametes and embryos of fish, and the study of cryo-impact on gene expression and sub-cellular structures. His is currently leading the development of cryo-banks of cells and tissues of UK fish species and also co-ordinating the collection and banking of material from critically endangered fish species worldwide as part of the Frozen Ark Project.



David Remsen (°1962) is a senior programme officer for the Global Biodiversity Information Facility in Copenhagen, Denmark. He works on the development of taxonomic infrastructure as a component of the GBIF network. His focus is on the development of taxonomic name services that include authoritative catalogues of common and scientific names of species and the application of these names, and information about their relationships, to the access and retrieval of information about species. He is the architect of the uBio Project in the USA and is a member of the EDIT Strategic Advisory Council.



Swen Renner (°1974) is Research Scientist at the Institute of Experimental Ecology at the University of Ulm. His research is mainly about biogeography and taxonomy of SE Asian birds and the impact of land use on bird communities. His work involves a wide variety of methods including museum collection work, field work as well as GIS and lab analysis.



Isabel Rey (°1961) is the curator of Tissues and DNA of the Museo Nacional de Ciencias Naturales of Madrid. Her research is focused on preservation of DNA and on methodology to obtain ancient DNA from classical zoological collection specimens. In addition, she is involved in designing standard protocols to obtain non invasive samples of living animals.



Klaus Riede (°1953) is Associated Researcher at the Zoological Research Museum Alexander Koenig, Bonn (Germany). His research focuses on neuroethology, bioacoustics and ecology of Orthoptera, with a strong focus on tropical species. He managed major biodiversity database projects, such as a global geo-database on migratory species (Global Register of Migratory Species) and a web-based multimedia database on Orthoptera specimens with song recordings. At present, he is working on cricket sensory ecology at the Smithsonian Tropical Research Station at Barro Colorado Island (Panama), with funds from the FWF Austrian Science Fund to the University of Graz.



Francesco Rovero (°1970) is the Curator of the Tropical Biodiversity Section at Italy's Trento Museum of Natural Sciences. Since 2002, he conducts research on diversity, ecology and conservation of rainforest mammals in the Eastern Arc Mountains of Tanzania, with a focus on census methods and ecological modeling of primates and ungulates. He works predominantly in the Udzungwa Mountains, where he helped establishing, and currently directs, the Udzungwa Ecological Monitoring Centre, a field station annexed to the Udzungwa Mountains National Park. He holds a PhD in Animal Ecology at the University of Wales in Bangor (UK) and published over 40 scientific papers and book chapters.



Yves Samyn (°1972) is employed by the Royal Belgian Institute of Natural Sciences in Brussels (Belgium) where he is coordinator of the activities of the Belgian National Focal Point to the Global Taxonomy Initiative. In this capacity he organises training for students and young researchers from developing countries in particular from Africa. To this end he has developed a web site focused on capacity building in taxonomy (www.taxonomy.be). He also is the co-founder and editor-in-chief of the capacity building journal *AbcTaxa*. His research expertise is on the taxonomy of sea cucumbers (www.echinodermata.be).



Jim Sanderson (°1949) is the founder of the Small Wild Cat Conservation Foundation, a Fellow of the Wildlife Conservation Network, a conservation officer of the Feline Conservation Federation, and one of the world's leading authorities on small wild cats. He has studied the Guigna, assisted his colleagues in the capture of the Andean cat, Margay, and Jaguarundi. With his Chinese and Tibetan colleagues, he obtained the first pictures of the Chinese mountain cat taken in the wild. He has a Ph.D. in mathematics and also wildlife ecology and conservation. He is the co-author of four books, and published more than 100 peer-reviewed articles.



Juliane Schaer (°1982) is a MSc student of biology at the Humboldt University in Berlin. Her main research interests are systematics, evolution and ecology of Chiroptera. During the past 4 years she has worked in the mammal collection of the Museum für Naturkunde in Berlin and also has been involved in field trips to Costa Rica and two small mammal surveys in Guinea. Currently, she is investigating host associations and evolutionary relationships of West African chiropteran blood parasites as part of her diploma thesis at the American Museum of Natural History, New York.



Ole Seberg (°1952) is Professor at the Laboratory of Molecular Systematics, Natural History Museum of Denmark, Copenhagen. His main research is in Molecular Systematics and Molecular Evolution especially on the phylogeny of the Monocotyledons and the evolution of the plant mitochondrial genome.



Mark R. Shaw (°1948) is the former Keeper of Natural Sciences and is now honorary research associate at the National Museums of Scotland, Edinburgh. He has published about 200 papers on parasitic wasps, particularly on their host associations, ecology and developmental biology, but also including some faunistic, taxonomic and phylogenetic contributions. The main focus has been on Ichneumonoidea, especially those parasitizing Lepidoptera and spiders, and to a much lesser extent Chalcidoidea.



Axel Steiner (°1960) is a free-lancing biologist with a background in archaeology who has worked on the Lepidoptera fauna of Germany at the Staatliches Museum für Naturkunde in Karlsruhe. He has a long-standing experience in collecting nocturnal Lepidoptera and in monitoring species for conservation projects. At the Staatliches Museum für Naturkunde in Stuttgart he was involved in the development and maintenance of taxonomic databases of butterflies. His interests include insect photography, the history of entomology and of entomological illustration, and he has done some taxonomical work on Noctuidae (Lep.)



Jerome Sueur (°1972) is Assistant Professor at the Muséum national d'Histoire naturelle, Paris. He is interested in systematics and biomechanics. One part of his research focuses on the acoustic behaviour of insects with a particular attention to mechanical auditory processes of cicadas. He is developing a new method to assess and compare local biodiversity through acoustic analysis. He is the maintainer of seewave, an R package dedicated to sound analysis and synthesis.



José Templado (°1957) is a Researcher of the Spanish Research Council (CSIC) at the National Museum of Natural History of Madrid. He is mainly interested in reproductive biology, systematics and evolutionary diversification of marine gastropods. He has been involved in many fieldworks and oceanographic cruises. His scientific research resulted in over 110 scientific publications (including papers in scientific journals, books and chapters of books).



Conny Thiel-Egenter (°1976) is co-owner of FORNAT, a consulting company for nature conservation in Zurich, Switzerland. She is involved in projects on plant conservation, vegetation ecology and protected area management. During her PhD in the EU-project IntraBioDiv, she gained broad experience in floristics, plant biogeography and phylogeography. She has special interest in arctic-alpine flora and ecosystems.



Simon Tillier (°1950) is senior Professor at the Muséum national d'Histoire naturelle in Paris, and the leader of EDIT. He studied taxonomy and phylogeny of Molluscs and was the curator of land and freshwater molluscs at the Muséum national d'Histoire naturelle from 1976 to 1990. During this period he collected invertebrates in various regions of the world, particularly in New Caledonia, spending in total several years in fieldwork. From 1990 Simon Tillier turned to molecular systematics and phylogeny, and set up and directed the molecular systematics facility of the Museum. In parallel he got involved in organization of research in systematics at International, National and Museum levels.



Mathias Tobler (°1975) is a research ecologist in the Botanical Research Institute of Texas. He works with the Andes to Amazon Biodiversity Program on research and conservation in the Peruvian Amazon, with a focus on the ecology of Amazonian ungulates. He has a strong interest in developing new monitoring techniques and quantitative methods for data analysis. He is also involved in the development of new computer software tools for managing and analyzing large amounts of biodiversity data. He holds a Ph.D. in Wildlife Sciences from Texas A&M University.



Kees Van Achterberg (°1948) he made forty years ago his first Townes type Malaise traps to collect Braconidae (Hymenoptera). Both in Europe and SE Asia he experimented with modified designs of the traps and methods to prepare specimens from 70% alcohol. During the 2007 RMNH-IEBR expedition to S Vietnam he used 35 Malaise and Schacht traps simultaneously. He published 300+ papers and books on the taxonomy, phylogeny, biology and biogeography of Hymenoptera (Braconidae, Heloridae, Stephanidae, Ichneumonidae (Hybrizontinae), Pamphiliidae, Cephidae, Argidae and Thynnidae) and works currently as senior researcher at the National Museum of Natural History (Naturalis).



Didier VandenSpiegel (°1961) is employed by the Royal Museum for Central Africa, Tervuren (Belgium) where he is senior researcher in the Department of Zoology, section Invertebrates, non-insects. He also is the co-founder and one of the permanent editors of the capacity building journal *Abc Taxa*. He has research expertise in the taxonomy of echinoderms and millipedes.



Alain Vanderpoorten (°1974) graduated as a bioengineer and did his PhD at the faculty of agricultural sciences of Gembloux (Belgium). After several post-doctoral research programs on the phylogeny and evolution of aquatic bryophytes at Duke University (USA) and diversification of Proteaceae in the fynbos of South Africa at Rhodes University (RSA), he is now a research associate of the Belgian Funds for Scientific research. His research presently focuses on the evolution of island endemism in bryophytes.



Jackie Van Goethem (°1943) is Honorary Head of the Department of Invertebrates at the Royal Belgian Institute of Natural Sciences in Brussels. He was responsible for the curation of a worldwide-recognised reference collection of molluscs and other invertebrates. He also was Belgium's National Focal Point to the Convention on Biological Diversity and the promoter of a major project funded by the Belgian Development Cooperation for capacity building in taxonomy and collection management for developing countries. He is the Executive Secretary of the King Leopold III Fund for Nature Exploration and Conservation which offers opportunities for biodiversity related fieldwork outside Europe. His research interests cover a.o. terrestrial snails and slugs from Europe, Africa and New Guinea.



Jose Luis Velasco (°1947) is a researcher at the Museo Nacional Ciencias Naturales (CSIC) in Madrid. He is a specialist in Rotifera.



Christian C. Voigt (°1966) is a Senior Research Scientist at the Leibniz Institute for Zoo and Wildlife Research and also a lecturer at the Humboldt University in Berlin (Germany). His main research interests are the behavior, ecology and physiology of mammals, in particular bats. He combines novel methodological approaches such as stable isotopes with conventional techniques such as behavioral observations or ecological surveys. His research focus has been in the New World with a strong focus on Central and South American bat assemblages, but recent projects have brought him in contact with the African continent as well.



Lee Weigt (°1960) is the Head of the Smithsonian Institution's Laboratories of Analytical Biology (L.A.B.) at the National Museum of Natural History. Since 1985 he has managed and directed molecular laboratories at several major museums, research institutes and universities. His current efforts are focusing on bringing together cutting edge biotechnology and bioinformatics tools to the Smithsonian scientists studying biodiversity, and on sharing information amongst research efforts. His personal research interest surrounds recently diverged species and species complexes.



Kurt Weising (°1954) is full professor at the University of Kassel. His research is focused on the phylogenetic reconstruction and evolution of plants. Research projects cover intra specific as well as higher taxonomic ranks and use traditional taxonomic (anatomy, morphology, etc.) as well as DNA-based molecular methods (e.g. micro satellites, DNA sequencing).



Gudrun Wibbelt (°1971) is senior veterinary pathologist at the Leibniz Institute for Zoo and Wildlife Research, Berlin (Germany). Her research focuses on pathology and infectious diseases of wildlife animals. She is particularly interested in diseases in bat species and is working on this subject since some years.



Thomas Wilhalm (°1965) is curator of Botany at the Museum of Nature South Tyrol in Bolzano/Bozen (Italy). He is interested in floristics and taxonomy (especially of alpine species and of Poaceae) and has acquired a broad experience in these topics during the project "Mapping the flora of South Tyrol" which he manages. Other activities include setting up herbarium BOZ, of a computerized database of floristic records and updating the Red List data of endangered vascular plants of South Tyrol. He is also involved in national and international projects concerning floristic mapping, molecular biogeography and taxonomy.



Friederike Woog (°1968) is curator of the bird collection at the State Museum for Natural History in Stuttgart, Germany. In 2003, she started a long-term study on site fidelity and biology of rainforest birds in Madagascar.



Holger Zetzsche (°1971) is a postdoctoral botanist at the Botanic Garden Botanical Museum Berlin-Dahlem and Germany. During the last 3 years he has been involved in the establishment of the DNA Bank Network. He is interested in long-term DNA storage, DNA based plant identification as well as systematics and evolutionary diversification of *Pulsatilla*. Although trained as a classical botanist he acquired a broad knowledge of molecular tools on various taxa of plants and algae.



Elke Zippel (°1968) is a researcher at the Botanic Garden and Botanical Museum Berlin-Dahlem, Germany and project manager for the National Seed Bank for Crop Wild Relatives in Germany. Her further interests include population genetics and phylogeography of rare plants in Central Europe, ecology and dynamics of bryophyte vegetation, as well as the flora and vegetation of the Canary Islands.

Previous titles in this series

Taxonomie des holothuries des Comores

Y. Samyn, D. VandenSpiegel and Massin C.
Abc Taxa Vol 1 - 2006

Détérioration des collections de coquilles

R. De Prins and E. Rour (traduction)
Abc Taxa Vol 2 - 2007

Taxonomy of the *Cryptocarya* species of Brazil

P.L.R. De Moraes.
Abc Taxa Vol 3 - 2007

Guia taxonomica de los anfibios de Cuba (with Audio CD)

L.M. Diaz and A. Cadiz
Abc Taxa Vol 4 - 2008

Introduction to the taxonomy of the amphibians of Kaieteur National Park, Guyana

P.J.R. Kok and M. Kalamandeen
Abc Taxa Vol 5 - 2008

Sri Lankan Seaweeds – Methodologies and field guide to the dominant species

E. Coppejans, F. Leliaert, O. Dargent, R. Gunasekara and O. De Clerck
Abc Taxa Vol 6 - 2009

The Bee Genera and Subgenera of sub-Saharan Africa

C. Eardley, M. Kuhlmann and A. Pauly
Abc Taxa Vol 7 - 2010

Manual on field recording techniques and protocols for All Taxa Biodiversity Inventories and Monitoring

Abc Taxa

If the Biosphere were a kitchen, this would be the cookbook every kitchen hand working in it would want to have. It is encyclopaedic in scope, without missing on detail where it's necessary.

The aim is to provide a guide to undertaking All Taxa Biodiversity Inventory+Monitoring (ATBI+M), with the laudable aim of accelerating the integration of taxonomic knowledge with biodiversity conservation and management. The ATBI+M approach has its critics, but there can be little doubt that the more information we can garner at the species level of biodiversity, the better we will be able to manage it as a whole. Warning against the complacency of one-time surveys there is advice on groups from whales to waterbears; slime moulds to forest trees, and everything in between. Particularly pleasing is the inclusion of a broad range of habitats, especially marine and freshwater. Some chapters are also on unexpected but important survey methods including bio-acoustics, camera trapping, and on specimen handling and preservation. Each chapter also has plenty of "how to's" for preservation and heaps of leads for identification – even in one case the advice on what sort of accommodation actually works in the field! And, as pioneered in Central America, reference to how para- and amateur taxonomists (increasingly called citizen science) must be part of the process if the exercise is to work.

Even if you are not doing an ATBI+M you will find this book useful, even fascinating. There is much much more in it than "just" collecting and survey advice. It may not seem bedtime reading, but just try it is my advice. Happy inventorying!.

April 2010

Dr. Peter Bridgewater
Chair of the UK Joint Nature Conservation Committee
United Kingdom