FIELD GUIDE

2018

MONITORING PROTOCOL FOR AQUATIC BIODIVERSITY OF RIVER GANGA

For Rapid Field Assessment

भारतीय वन्यजीव संस्थान
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Preface

The objective of this field guide is to communicate field friendly standardized protocols for data collection for the frontline staff and officers of the forest department. The data when analyzed would provide a scientific basis for assessing the status of aquatic biodiversity of river Ganga and their habitats.

This field guide will serves as cook book for data collection protocols on biological diversity of Ganga River. The Ganga River Basin is one of the largest living river systems in the world, and has significant economic, environmental and cultural value in India. It supports more than 25,000 floral and faunal species and serves as a lifeline for the population of over 500 million people. The Ganga Basin is also a home to a wide range of aquatic species, including the Gangetic dolphin, three species of otters *viz.* the smooth-coated otter Eurasian otter and the small clawed otter, the critically endangered Gharial, mugger or Indian marsh crocodile, Estuarine crocodile and at 13 species of freshwater turtles including critically endangered and several species of fish such as critically endangered Gangetic shark, Gangetic stingray, Mahseers, Hilsa and several species of endemic freshwater crabs.

Understanding the importance of this riverine system and its biodiversity this protocol has been designed to analysis the population status of Mammals, Aves, Reptiles, Amphibians fishes and river vegetation data using document standardized methods.

Contents

INTRODUCTION

Reliable, standardized and replicable methodologies for quickly assessing key ecosystem biodiversity in the field are essential for conservation planning and decision-making at the local to regional scale at which most threats occur. Rapid field assessments are a cost-effective solution to this problem, providing data in a timely manner to address a wide range of conservation needs, and in particular to establish a baseline that can be used to detect changes over time. A great deal of high level methodological guidance exists, but most lack practical implication details. A literature describes relatively comprehensive sampling methods but do not focus on a core set of standardized methods, making it difficult to decide which protocols to adopt. Other publications are available with lengthy, detailed guidance on sampling individual taxa. This is the drafted protocol that focuses exclusively on a concise, practical set of standardized protocols for a wide range of taxa with species reference on aquatic biodiversity. This is no simple task. Many scientists tend to employ their own individualized, often opportunistic, approaches for finding as many species as possible in a short time, sometimes honed through decades of personal experience. These contributions are invaluable; yet do not address many conservation requirements. While not intended to replace these methods, the identification of a core, at-a-minimum set of standardized methods, including innovative and automated approaches where applicable, is of great importance for making the results of rapid surveys comparable and replicable across sites and over time. New technologies and automated equipment make rapid surveys increasingly more cost-effective and unbiased. These methods also move beyond presence-absence records to record relative or absolute abundance, which is crucial for assessing threat and monitoring change. Typically, rapid assessments require at least one week per site. A critical and often unanswered question in baseline assessments is how to know when sampling effort is sufficient. We have addressed this question with representative species accumulation curves and analyses in each individual chapter. Regional differences in ecosystems, climates, and Evolutionary histories also mean that methods for some taxa need to be tailored to particular geographies.

The adoption of standardized methods provides the following benefits:

- Methods can be more easily replicated when the same site is sampled at a later date, which is especially important if different researchers are involved, making it possible to understand how biodiversity has changed over time
- Biodiversity data from a particular site can be placed within a regional or global context because it can be easily compared with data from other sites where the same methods were employed
- Sampling completeness can be estimated, which allows interpretation of how many species occur at a given site. Estimating sampling completeness relies on statistical approaches to determine the actual number of species occurring at a site based on standardized sampling effort
- Standardized sampling provides population-level abundance data as opposed to other opportunistic sampling that can yield only presence-absence data; the former is much more powerful for understanding changes in biodiversity over time and for identifying rare species that may be more vulnerable to environmental change
- Standardized methods allow even amateurs to be trained effectively in many cases, reducing the dependence on only a handful of experts globally. However, this book is still targeted towards professional biologists and is not intended to provide a sufficient level of detail for novices to apply in the field

The focus of this book is on tropical terrestrial and freshwater ecosystems worldwide, although most methods should be applicable in temperate zones as well. It is not possible to sample all taxonomic groups during a rapid survey. In this book, we describe methods for major taxonomic groups (plants, vertebrates), as well as a select set of invertebrates that represent costeffective indicator taxa and play important ecological roles. This book represents a consensus of multiple experts for each taxonomic group, including intensive peer review. We expect that a future edition of this book will include methods for marine taxa, various ecosystem services, as well as social assessments.We do not include a separate chapter on analytical approaches or data management, as these are already well covered in other publications (e.g., Hill et al. 2006; Sutherland 2006; Eymann et al. 2010). Other organizations and institutions have used similar rapid assessment approaches to achieve tremendous conservation outcomes. We hope this protocol will help to unite the broad range of institutions and researchers who continue to advance knowledge building through field assessments

The Ganga River Basin is one of the largest living river systems in the world, and has significant economic, environmental and cultural value in India. It supports more than 25,000 floral and faunal species and serves as a lifeline for the population of over 500 million people. The Ganga Basin is also a home to a wide range of aquatic species, including the Gangetic dolphin (*Platanista gangetica*), three species of otters *viz.* the smooth-coated otter (*Lutrogale perspicillata*), Eurasian otter (*Lutra lutra*) and the small clawed otter (*Aonyx cinereus*), the critically endangered Gharial (*Gavialis gangeticus*), mugger or Indian marsh crocodile (*Crocodylus palustris*), Estuarine crocodile (*Crocodylus porosus*) and at 13 species of freshwater turtles including critically endangered *Batagur kachuga* and several species of fish such as critically endangered Gangetic shark (*Glyphis gangeticus*), Gangetic stingray (*Himantura fluviatilis*), Mahseers (*Tor* spp.), Hilsa (*Tenualosa ilisha*) and several species of endemic freshwater crabs. Within the Ganga River system, 143 different freshwater fish species, comprising 11 orders, 32 families and 72 genera have been recorded (Sarkar et al., 2011). In addition to these species, obligate aquatic species like waterbirds and island nesting birds are important component of the Ganga River Basin. It also endures numerous diverse ecosystems, from the alpine forests to the mangrove forests and saline mud flats. Despite its importance, the aquatic wildlife of the Ganga basin including the main stem Ganga River is under stress due to reduction in water levels, pollution, over exploitation of riverine resources leading to great threat to the biodiversity and environmental sustainability of the River Ganga, with detrimental effects important habitats for wildlife.

The Ministry of Water Resources, River Development and Ganga Rejuvenation, Government of India has initiated the National Mission for Clean Ganga (NMCG) with a comprehensive approach to restore the aquatic environment of Ganga through four different sectors, *viz.* wastewater management, solid waste management, industrial pollution and river front development. It has also developed a comprehensive strategy to restore the biodiversity values of the Ganga River.

In order to address the main problems and issues of River Ganga it is important to develop protocol consists of reliable, standardized and replicable methodologies for quickly assessing the key biodiversity status.

These rapid biological assessments will further help in identifying the major issues and challenges threatening the survival of many important biological species. These assessments

also provide cost-effective solution by providing and forming the baseline data that can be used to detect changes over time.

A great deal of high level methodological literature and guidance exists on aquatic biodiversity, but most lack practical details. Few books describe relatively comprehensive sampling methods but do not focus on a core set of standardized methods, making it difficult to decide which protocols to adopt. Other publications are available with lengthy, detailed guidance on sampling individual taxa. We believe this is the first book that focuses exclusively on a concise, practical set of standardized protocols for aquatic biodiversity.

This protocol also outlines and intended to document all scientific methodologies for rapid assessment of aquatic biodiversity including innovative and automated approaches where applicable, is of great importance for making the results of rapid surveys comparable and replicable across sites and over time. New technologies and automated equipment make rapid surveys increasingly more cost-effective and unbiased. This protocol outlines the rationale, sampling design, and methods for monitoring important biodiversity of River Ganga.

It is not possible to sample all taxonomic groups during a rapid survey. In this book, we describe methods for major taxonomic groups.

Introduction

Large mammals are considered good bioindicators of intact tropical landscapes and have therefore been increasingly used in large-scale monitoring programs worldwide (Ahumada *et al*. 2011; Luzar *et al*. 2011; Nobre *et al*. 2013). They play vital roles in ecosystem structure and functioning, participating in different trophic levels in food webs, contributing to herbivore regulation, and acting as important seed dispersers for many tree species (Terborgh 1992). They are also a vital economic resource for local human populations through their use as food, pets, artefacts and tourism (Peres 2000; Costa *et al*. 2005). Indeed, mammals comprise an important source of protein and income to local communities, especially the large-bodied species given their great amount of meat (Redford 1992; Peres 2000). Moreover, they are widely hailed as regional conservation icons (e.g., pandas), as many species are charismatic and benefit from popular sympathy, which contributes to promote conservation to the wider public (Cuaron 2000; Dirzo *et al*. 2014). Regardless of their appeal, many mammal populations have gone extinct and many others are declining, requiring surveys and monitoring that can inform conservation action to hinder continued population declines.

Core Methods

Sampling Protocols Line-transect preparation: within each major habitat type (site), three 4-km long and 1-m wide linear transects should be cut before the start date of surveys. The number of sampling sites and distances to each other may have to vary according to the total area of the study landscape. Ideally, transects should be established at least a week before the start of rapid assessments so that human disturbances do not affect mammal behavior and results. At this step, transects should be measured (using a Hip-Chain or a 50-m tape) and marked (using a biodegradable flagging tape) every 50 m. Within each sampling site, transects should be separated from each other by at least 1 km, and their location should take into account accessibility, including the existence of rivers, streams and topography, that might hamper the surveys. It is best to open transects more than 300 m from the base camp to avoid biased data due to any species behavioral responses to camp activity. Shorter transects may be necessary in fragmented forest sites where space constraints prevent long trails. Within fragmented forest landscapes, the length and arrangement of transects should consider both area and shape of each forest patch, aiming to cover a representative area (50% of a

patch would be adequate). Prior to the start of the surveys, a field sheet should be prepared to enable data records during the data collection.

Diurnal line-transect census :Two observers, preferably one trained researcher accompanied by a local inhabitant with knowledge of species present, should walk at a constant speed (~1 km/h), with brief stops (10 s) every 500 m, along each of the three transects established at each site (Peres 1999; Peres and Cunha 2011). Transects should be walked in both directions, for a total of 24 km of sampling effort per day (3 transects walked simultaneously x 8 km). In savannas, surveys can also be conducted using a vehicle, at approximately 10 km/h. Surveys should be conducted in the morning (~6:15 – 10:30) and afternoon (~14:00 – 17:30), and discontinued during rainy periods since these can affect results. At the start of each survey, the lead observer should record the date, transect identity, name of observers, general weather condition (sunny, overcast or cloudy) and start time. Observers will then start walking along the transect, keeping a distance of ~15 m from each other, looking for target species in all strata (in case of forest habitats) and on both sides of the transect Upon a visual detection event, observers should record: the time, species name, number of individuals, sighting location along the transect, and the perpendicular distance from the animal (or first detected individual, in case of groups) to the trail, which needs to be accurately measured (Hip-Chain or a 50-m tape; Fig. 1 and Appendix I). It is important that the observers see or hear the animal(s) before they detect the observers – otherwise, the perpendicular distance may be inflated, directly affecting density estimates. For each detection event, observers should remain on the transect line and spend no more than 10 minutes to count individuals and record the data. The end time of each walk should be recorded at the end of each morning and afternoon survey. In order to minimize biases related to the probability of detection, each pair of observers should be rotated on a daily basis between different transects. The number of sightings per km walked (sighting rate) should be used as a measure of abundance (both for groups and individuals), and density estimates can be calculated using specific programs such as Distance (Buckland et al. 1993). Probability of occupancy can be assessed by using a matrix of presence absence data per survey for each species, using programs such as Presence (Hines 2006; see Box I for parameter terminology).

Indirect surveys: Concomitantly to the diurnal line-transect surveys, the same two observers should search for any indirect evidence of target species along and up to 5 m from the transect. Local field guides should be used to identify mammal tracks. Acoustic records of

identifiable species could also be recorded. The field sheet can be the same as that used to conduct linetransect surveys, but the perpendicular distance is not recorded as this methodology cannot discern accurate density estimates. However, indirect surveys may enhance the number of mammal species recorded within a site, and enable occupancy estimates that can be used to detect changes over time.

Selecting sampling sites: The location of sampling sites should cover a representative area of the study landscape. A first required step is to obtain satellite images of the study landscape to acquire knowledge of habitat distribution, existence of rivers, local villages and other site characteristics. Next, a visit to the area should be performed prior to the survey in order to select the sampling sites. This visit should be used to assess logistical challenges (feasibility for surveys), habitat heterogeneity (focus on the most representative habitat types), anthropogenic disturbance (depending on the goals of the survey, which may be to focus on intact sites, or to assess disturbances such as logging, fire and hunting), and accessibility (if the access occurs by boat, transects should start close to rivers and streams in order to reduce time walking prior to each survey). All transects should not traverse aquatic realms inaccessible by foot. Effort required – A minimum of seven days of two-way surveys (i.e., morning and afternoon) along
each transect at each sampling site is required. This will provide a total of 168 km of cumulative effort. This effort is expected to provide robust species richness for each sampling site – previous studies in Neotropical forests recorded up to 93% of all species (extrapolated richness) considering a total survey effort of 80-90 km (de Thoisy et al. 2008). However, some cryptic species are difficult to detect even with higher sampling efforts, although abundances can be obtained with such effort, and provide a good proxy of communities status. For occupancy models, one week of surveys will provide 14 ‘visits’ on the presence-absence matrix, which is potentially adequate for analyses of site occupancy for most species. For density estimates, however, a minimum of 40 detection events are recommended for robust estimates, although 20 sightings may provide sufficient estimates (Peres 1999). If small sample sizes were obtained at the end of rapid surveys, data from different sites can be pooled together to enable density estimates using the Distance software. Sighting rates can also be calculated and compared among different landscapes independent of sample size.

Camera Traps : Camera trapping is an excellent tool that helps to avoid the difficulties described above and is complementary to line transects for assessing and monitoring

terrestrial vertebrate communities (O'Brien 2008; O'Brien et al. 2010; Tobler et al. 2008, Ahumada et al. 2013). Camera traps have several advantages: they are automated and standardized, helping to eliminate individual sampler bias and reducing researcher hours required in the field; and they operate 24 hours per day and can be left in place even when researchers are not present, increasing detection rates even for highly elusive species. Arrays of camera traps act as visual sensor networks to detect and monitor the variation of terrestrial vertebrate relative abundances in space and time (Kays et al. 2011), where the rate and the proportion of points at which species are photographed (occupancy) can be used as an indicator of their abundance. Camera trap data can also be used to estimate population densities (Rowcliffe et al. 2008).

Live-trap transects Although there are many sampling protocols, such as those discussed later in supplementary techniques, the core method for standardized rapid surveys is defined as a line transect of large (9" x 3" x 3.5") aluminum box-style live traps (e.g., Sherman LFA Folding Trap, Longworth). An outline of a trapping procedure is given below and ideally suited for 2 experienced biologists. If personnel and budget is not constrained, the core method can be increased at a percentage that facilitates scaling for standardization purposes and supplementary techniques are highly recommended.

- ✓ Along each transect, 2 traps set every 10 meters (e.g., 1 trap every 5 m or 2 traps at a station every 10 m to standardize for different methods of trap setting).
- ✓ 3 transects of 200 m or 2 transects of 300 m with transects radiating out from camp to maximize coverage; unlike relatively intact habitats, for areas that are more disturbed and to avoid noise disturbance, transects may be placed at a considerable distance from camp.
- ✓ Minimum of 120 traps per night of sampling effort, but whenever logistically possible should be increased by a convenient factor (e.g., 50% or 100%) for standardization purposes among studies. For rapid surveys, each locality should be sampled for at least 5 nights or ideally for 1 week. Some preliminary inventories of a habitat recommend more trap-nights of effort (Fraser *et al*. 2003, Jones *et al*. 1996), but this may not be feasible for monetary reasons (see Box 1 for cost of traps).
- ✓ Whenever possible, traps should be set off the ground to sample arboreal species traveling on vines, tree trunks, or low branches (flagging tape or twine can be used

to secure the traps to the branch or vine) because this micro-habitat will not always be available at every potential trap site; for example, if 2 traps are set every 10 metres then one can be set on and the other off the ground, or alternate traps on and off the ground every 5 metres when possible.

- ✓ Traps on the ground should be set in areas where animals may be foraging, such as at the base of large trees, or along likely corridors of movement, such as along tree falls, and on top of logs.
- ✓ In closed forested habitats, animals will not be typically foraging in open areas, and will be wary of predators and keep closer to trees or logs for cover. Consequently, traps should be set with the open door facing trees or logs at about the same distance as the width of the door (e.g., 3" for large Sherman traps)
- ✓ In open grassland habitats, traps should be placed along possible foraging runways on the ground or near potential cover such as shrubs or solitary trees
- ✓ Check that the trigger is properly set on each trap by testing the treadle sensitivity with your hand and adjusting the trigger accordingly
- ✓ Every trap is marked by a piece of 8" flagging tape numbered sequentially with a waterproof marker and tied to nearby vegetation.
- ✓ Traps should all be baited, for example with raw unsalted unshelled seeds such as sunflower or a mixture of rolled oats, peanut butter, honey, bacon fat, etc.; if seeds are used, scatter a few (not many, ~12) in front of the trap and leading into the back of the trap; if a mixture bait is used, it should be placed on a piece of paper at the back of the trap to make removal and cleaning easier; seeds are recommended as the default bait as this is usually readily available in food markets; a secondary bait is dried corn kernels or rice. In the tropics the oily, shaved-off pericarp of oilpalm nuts (Elaeis guineensis) and bananas are also good bait, either alone or in the mixture with rolled oats, especially for marsupials.
- ✓ Trap lines should be checked in the early morning before it gets too hot or before ants discover the animals in the traps. In addition it is suggested to check traps, once or twice later in the day, if they are left open for diurnal species.
- ✓ In temperate or cooler regions, bedding material such as cotton should be put in the trap.
- ✓ Traps should be rebaited if heavy rain has spoiled the bait or bait has been eaten by ants, which may be a daily occurrence in some areas; if this becomes a

problem, traps can be baited in the late afternoon. Alternatively, bait could be wrapped with cheesecloth to retard ant activity.

- ✓ Normally traps can be left open during the day for possible diurnal mammals, but if by-capture of non-target groups such as reptiles is detrimental then traps can be opened and baited in the late afternoon
- ✓ If a trap has caught an animal, it should ideally be processed (examined, marked and released) on the spot. Alternatively, the trap should be replaced by a spare trap or by a small piece of flagging and the trap (with the animal inside) brought back to camp for specimen processing and data recording (the flagging acts as a reminder of where the trap should be reset the next day). The removed trap can also be identified by writing

Monitoring of River Dolphins

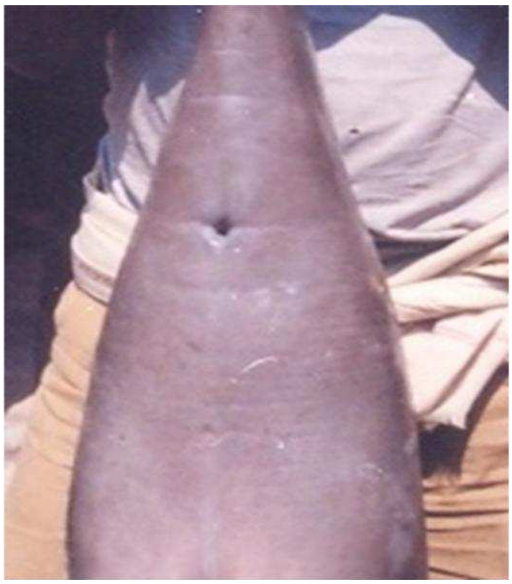
- GANGES RIVER DOLPHIN (*Platanista gangetica gangetica*)
- INDUS RIVER DOLPHIN (*Platanista gangetica minor*)
-
- IRRAWADY RIVER DOLPHIN (*Orcaella brevirostris*)

- Ganges dolphin – endangered, Schedule I
- Indus dolphin – endangered, (Not listed)
- Irrawady dolphin – Critically Endangered, Schedule I

Why should the River Dolphin in India be conserved

- National Aquatic Animal of India
- Baiji extinction in China
- Precarious status in Nepal and Bangladesh
- India is last stronghold with extant populations in Ganges – Brahmaputra and Re-discovery in Indus basin
- An excellent indicator of riverine ecosystem health
- Unique riverine species with echolocation abilities
- Friend of fish and fishermen

Identification Features



Male

Female

Threats for River Dolphins

- Fisheries By-catch mortalities

- Dams and Barrages

- Loss of habitat
- Pollution
- Developmental projects
- Lack of conservation focus
- Threat of violence

Survey for estimation of abundance

- Nobody can count all animals in a population ever because of ecological reality & human limitations.
- Hence, statistical estimation of uncertainty is the way forward.
- Methods of conducting population surveys of Ganges river dolphins have been hitherto dominated by what may be grouped as 'total-count' methods.
- Total count methods does not really tell us much even about population trends.
- Methods of conducting population surveys of Ganges river dolphins have been hitherto dominated by what may be grouped as 'total-count' methods.
- 'Total-count' methods' - fundamentally based on the assumption that almost all dolphins surfacing within a specified time period will be counted by observers provided sufficient time is available to detect surfacing individuals.
- Total count methods, typically done by one observer team, employs several survey modes: (i) Point counts - non-random, (ii) Transects - non-random (iii) Boat- or shore-based surveys (iv) Upstream- or downstream surveys.

Issues in counting an elusive aquatic mammal

- Counting Ganges river dolphins well enough is not an easy task by any means as both the dolphin and its riverine habitats are incredibly complex to observe properly.
- Dolphins - highly elusive, with very little observable surface behavior, very short surfacing time.
- River waters - do not allow for in-water visibility.
- Thus the dolphin would be 'available' to detect only for a fraction of a second.
- Commonly used methods such as line-transects or Distance Sampling (DS) assume that detection of animals on the transect line is certain, denoted conventionally as $\hat{g}(0)=1$.

- Estimation of the abundance of aquatic diving mammals like cetaceans involves assessing the issues of availability bias and detection probability, or perception bias.
- Assessing the issues of availability bias and perception bias requires the estimation of two probabilities:

  i) the probability of animals being available for detection (i.e. when they surface above the water) &

  ii) the probability of being detected by the observer when they are available for detection.

Because of these issues, the assumption of certain detection on the line does not hold in the case of cetaceans, i.e. $\hat{g}(0)<1$.

- Further away from the line, probability of sighting an animal might be conditional on multiple covariates related to observer attention, weather conditions and animal behaviour.

So, such detection is often strongly subject to observer error.

In search of optimality and efficiency along with robust estimation:

- An ideal river dolphin survey must address:

i) the issue of imperfect detection some way or the other, such that bias about estimates may be minimized,

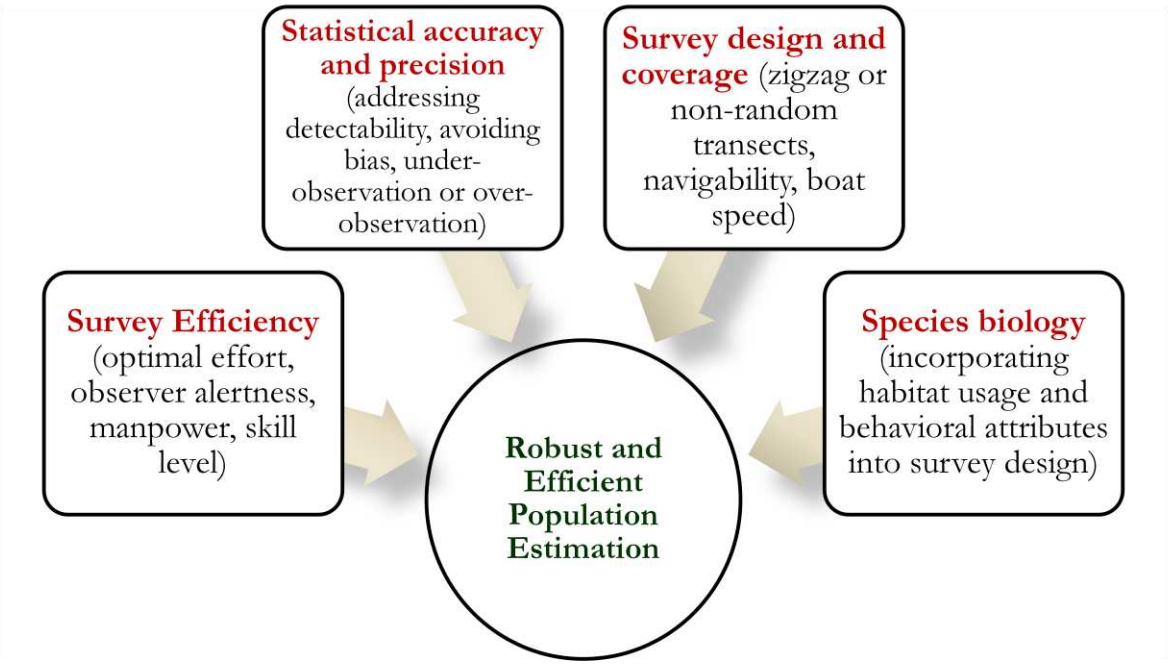
ii) needs to be efficient in terms of ensuring high observation quality even over large survey regions, by being quick and highly rewarding to observers to maintain their alertness,

iii) must also take into account sources of variation in observability: weather condition, time of day, type of habitat and so forth, &

iv) must clearly define the study region and the relevance of the extrapolated estimates bound by area.

None of these tasks are handled by current upstream surveys based on single-observer teams, however efficient they may appear even to experienced observers.

Components of an ideal and optimal survey with the objective of robust and efficient population estimation



Relative advantages and disadvantages of different survey methods used for Ganges River dolphins

Total Count Method:

Advantages: Low skill requirements, useful in doing one-off initial surveys.

Disadvantages: No estimation of uncertainty about population size provides only counts with no confidence interval, therefore no inference about population trends can be drawn, faulty ecological assumptions, almost impossible to compare data with another survey.

Direction: Upstream surveys preferable over downstream surveys for sampling a higher proportion of the population of interest.

Line transect Survey:

Advantages: Estimate detection probability based on distance of sightings, designs suited to zigzag or non-random sampling, allows better coverage of habitats, provides strong inference about density and detection variations, also flexible and can include covariates.

Disadvantages: Very difficult to meet assumptions of Distance Sampling Theory in field, surveys often impracticable due to channel braiding etc. and navigation problems, need high

skill requirements and modeling familiarity, complex designs and high scope for observer error, limitations in extrapolation.

Direction: For zigzag random surveys survey direction might not be critical, for spatial non-random surveys upstream surveys may be preferable.

Double observer survey

Advantages: Relatively free of design issues, model-based robust estimation of abundance possible, clear estimation of abundance and detection probability, easy to calculate estimates at basic level, can include covariates very easily, relatively moderate skill levels needed, surveys can be highly efficient, widely applicable even over large survey regions.

Disadvantages: More trained manpower needed, two observer teams to organize, need customized boats with constructed platforms.

Direction: We recommend using downstream surveys over upstream double-observer surveys, for higher efficiency, optimal and fatigue-free sampling, and very good resultant estimates.

Survey efficiency, optimality and cost-effectiveness of double-observer downstream surveys when compared to other methods

Survey method (for 65 km stretch)	Days &hrs. required	Range of survey cost (USD)	Survey efficiency & observer fatigue	Remarks
Downstream Total Count	**1, 6-8**	**75-80**	**Low, Low**	**Inefficient also because of being split over 2-3 days**
Upstream Total Count	**2, 15-17**	**155-160**	**Low, High**	**Inefficient, extremely tiring and timed over whole-day, outcomes hardly useful**
Upstream Double-Observer surveys	**3, 16-19**	**175-190**	**Average, High**	**Some improvement in estimates, but extremely tiring and timing over the whole day**
Downstream Double-Observer surveys	**2, 7-8**	**60-80**	**High, Low**	**Consecutive days, surveys only at time of highest dolphin activity, so highly rewarding**

From design-based to model-based approaches: enter double-observer surveys

- In river-scapes where survey designs with random line-transects cannot be implemented, capture-recapture methods provide an alternative to design-based transect-line methods.
- In double platform surveys with independent observer teams, where sighting data from each platform, representing an independent capture occasion, can be used in a two-sample capture-recapture framework for estimation of abundance, using the simple, canonical Lincoln-Petersen estimator or the Chapman's bias-corrected estimator.
- Smith et al. (2006) used double-platform methods with visual surveys for freshwater cetaceans, and capture-recapture methods for abundance estimation, in the Bangladesh Sunderbans; estimating the abundance of Ganges river dolphins at 196-225 (SE 24.9) with a survey effort of 1561.5 km.

Compiling Traditional Ecological Knowledge (TEK) of Veteran Fishers about Fisheries and Riverine Biodiversity

- The conservation and management of ecosystems and wildlife requires a strong scientific understanding of history.
- In the lower Gangetic plains, the construction of the Farakka barrage was a watershed that led to ecosystem-wide declines in river fisheries.
- Traditional fishermen in the lower Gangetic floodplains have had a long relationship with the hydrology, habitats and seasonal variations in fish resource availability.
- Traditional Ecological Knowledge-TEK is the vast store of accumulated ecological information & comes from years of fishing experience in the riverscape.

Monitoring protocol for otters

Surveying and monitoring otter populations

***Lutra perspicillata* – Smooth-coated otter**

Size: Length 1067-1300 mm

Weight: 7-12 kg

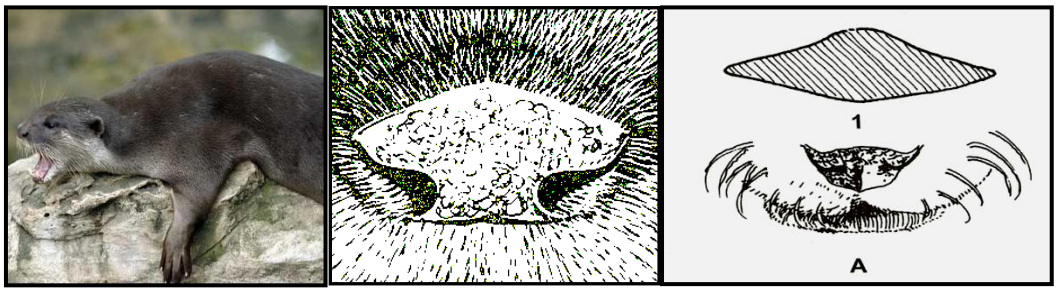
Feet: large, webbed and thick, claws strong

Tail: tapered, with slight flattening at sides

Rhinaruim: bare, dusky with peaked upper margin

Face: cheeks light gray, sharply demarked from color of upper parts

Hair texture: velvety smooth



***Lutra lutra* – Eurasian otter**

Size: Length 1020-1370 mm

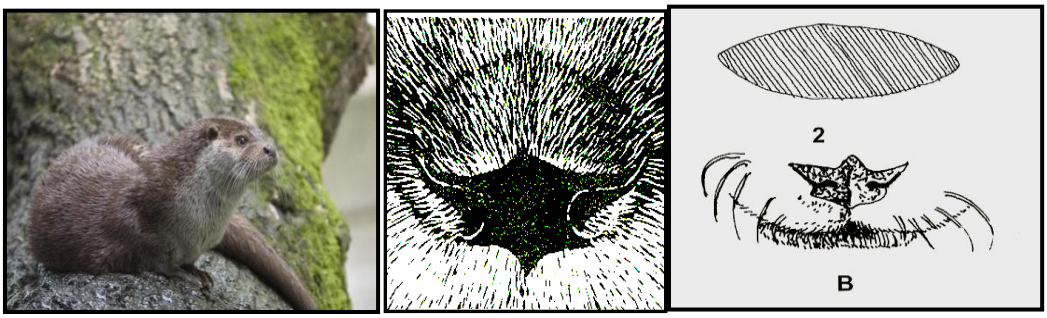
Weight: 7.00-12 Kg

Feet: well webbed, claws strong

Tail: thick at the base, tapered

Rhinaruim: naked, large, shield shaped

Hair texture: moderately coarse



***Anoyx cinerea* – Asain Small Clawed Otter**

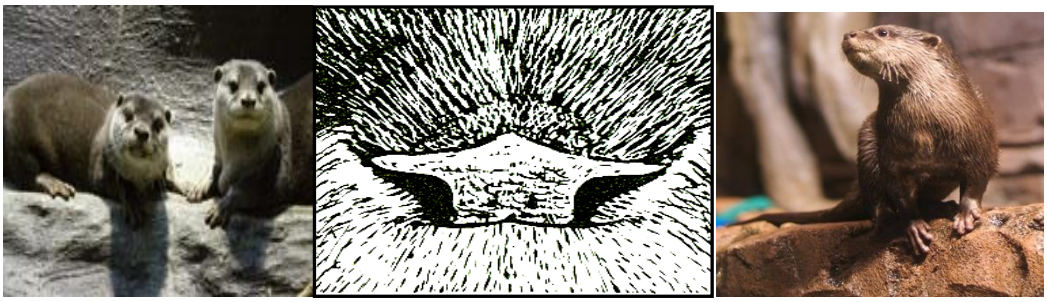
Size: length 652-939 mm

Feet: narrow, webbed only to last joint of toes; claws blunt, peg-like, rudimentary

Tail: tapered, not unusual

Rhinaruim: pink or dusky

Hair texture: not unusual, velvety



Otter Survival

- Relatively undisturbed wetland habitat
- Protection from poaching/persecution
- Abundant of prey species. Generally 100 kg of prey biomass supports 1 kg of predator biomass
- At least 17 km of river stretch supports one family of smooth otter
- Adequate vegetative cover along the shore line as escape cover for movement and dispersal
- Presence of denning, resting and grooming sites

Monitoring

- Monitor population trend to assess the status of otters and threats based on standard methods
- Ensure adequate prey base by providing protection to otter habitats
- EIA of all development projects affecting wetlands and otter habitats
- Control poaching and persecution
- Legal protection should be provided to Otters
- Undertake awareness campaigns to conserve wetlands and their obligates species such as otters
- Involve local communities in wetland conservation

Designing field methodology

- Stratify survey area into different zones or grids
- At each site, search a belt of 15-25 m area of shoreline up to 500-1000 m
- Search intensity can be increased by searching specific plots (100 x 25 m) laid in regular intervals (500 m)
- Habitat variables to be recorded: Types of sign, substrate characteristics, shore line vegetation status, water depth, water current, river width, otter seen, associated fauna, anthropogenic factors, visible pollution
- Signs may be removed to avoid duplicate count or for occupancy survey

Data sheet

Site #	GPS location	Altitude	Habitat type	Type of sign	Substrate type	Shore line vegetation	Water depth	Water current	River width

BOX 1: Examples of Equipment and Supplies

Core Methods:

• Sherman large LFATDG Folding Live Trap

• Alternatively for tropical environments with long-tailed rodents such as kangaroo rats: Sherman extra-large XLK Folding Live Trap

• Bait (raw, unsalted, unshelled seeds; e.g., sunflower seeds from local market; oil palm nuts in the tropics)

• Flagging tape (1 roll)

• Waterproof marker

Secondary Methods:

• Forestry Suppliers Museum Snap Trap

• Sherman XLF15 Folding Live Trap

• Tomahawk 204 Single Door Collapsible Live Trap

• 201 - Collapsible Trap - Chipmunk/Gopher/Rat Size or Tomahawk 203 Double Door Collapsible Live Trap

• Pitfalls – 11 20-liter buckets, plastic sheeting (100 m X 0.5 m), shovel, machete, staple gun or hammer and nails for securing plastic to wooden stakes that can be cut from staplings

Specimen Preparation:

• Field notebooks and catalogue sheets (water resistant paper)

• Indelible ink pens (e.g. Pigma Micron or Rotring Tikky Graphic) and pencils

• 12" ruler and/or tape measure for larger species

• Pesola scales (30g, 100 g, 500 g and 1000g)

• 2-ml tissue vials

• 95% ethanol

• Formaldehyde (dilute to 10%)

• Anesthetic (Isoflurane, Halothane etc.)

• Forceps (fine tipped)

• Scissors (fine tipped)

• Cotton tags for alcohol and dry specimens

Data Sheet

Observers: _____ Site:_____ Transect:_____

Date: ___/____/____ Start time:_____ Final time:_____ 

Time	Species	Record type	N inds	Location	Distance	Obs

Kumar Ankit and Gopi G.V

Introduction

What are Waterbirds?

According to the Ramsar Convention waterbirds or waterfowls are "birds ecologically dependent on wetlands" (Kumar et al. 2005). The waterbirds are considered to be a significant biological resource and are excellent indicators of the health of Wetland ecosystems (Dynesius and Nilsson 1994). The groups of birds are "wetland specialists" and consist of species with diverse varieties of morphological characteristics and habits. Globally there are 871 species of waterbirds identified so far (Wetlands International, 2012). Waterbirds have many intriguing features in their biology that includes long-distance migration, colonial breeding, and congregation behavior (Burnette 2016). Though waterbirds are one of the well-studied groups of birds, the "hows", "whys" of many interesting behaviors like colonial nesting continues to evade scientists and continues to remain an evolutionary puzzle. We still have much to learn about this unique group of birds about their social and sexual behaviors, population dynamics, movement patterns, the impact of toxicity, managing infectious diseases and anthropogenic effects on species and their spaces, etc. Diverse wetland types are distributed across all the biogeographic zones in India (Islam and Rahmani, 2008). Which includes glacial lakes, rivers, streams, marshes, jheels, saline expanses, floodplains, estuaries, intertidal marsh, intertidal swamps, intertidal mudflats, lagoons, humanmade reservoirs, irrigated agricultural lands, aquaculture ponds and salt pans, etc. These diverse wetland habitats support 243 species of waterbirds belonging to 34 families in India. The Ramsar wetland type M includes all the permanent river, streams, creek including waterfall (Islam and Rahmani, 2008). The river ecosystem encompasses river channels and its floodplains and forms a diverse mosaic of habitats with the riparian area at the transition zone between land and water. These linear ecosystems provide an important sheltering, feeding and breeding area for waterbirds and provide significant stopover sites and refugia (Forneman et al. 2011).

What is Monitoring?

Monitoring refers to all sorts of *systematic* and *repeated* assessments, observations or surveillance of processes. Data are evaluated by comparing against so-called *baseline* data originating from the first observation series. The purpose of monitoring is to control a process through intervention based on the data evaluation. In the field of ecological monitoring (such as of waterbirds), the data lead to interventions concerning the management and conservation of species or habitats.

Why monitor waterbird?

Monitoring waterbirds show the change of population sizes over time. These changes may be caused by a plethora of factors, such as food availability in the breeding areas, predation, hunting pressure along the migration route, or environmental pollution. Waterbirds are well-known indicators of the quality of certain types of wetlands. A powerful tool which makes use of this characteristic is the so-called 1% criterion, whereby any site which regularly holds 1% or more of a waterbird population qualifies as a wetland of international importance under the Ramsar Convention on Wetlands. The 1% criterion has been adopted by the European Union to identify Special Protection Areas (SPAs) under the Birds Directive. It is also used by BirdLife International in the identification of Important Bird Areas (IBAs) in wetlands throughout the world.

Why is Ganga Important for waterbirds?

River Ganga is one of the significant river systems in India which provides a mosaic of habitat to different taxa. It flows through five states, and the length of the river is nearly about 2525 Km. National Mission for Clean Ganga – Wildlife Institute of India (NMCG- WII) "Biodiversity conservation and Ganga-Rejuvenation project aims at science-based aquatic

species restoration program. The conservation of these birds of the Ganga River is a part of the Ganga rejuvenation.

The Gangetic basin supports 177 species of these birds including wetland, riverine and terrestrial species. Some iconic and globally threatened the Ganga River. These species are indicators of the healthy river ecosystem, however, are vulnerable to hydrologic alteration, climatic shifts, and anthropogenic inference. River Ganga provides habitat to numerous waterbirds including globally threatened bird like Black-Bellied tern *Sterna acuticauda*, Sarus Crane Antigone antigone, Indian Skimmer *Rynchops albicollis* and River Tern *Sterna aurantia* harbors the river Ganga in different stretches. It also hosts numerous wintering migratory waterbirds. Monitoring wildlife species including the waterbird in this river would be useful to detect overall management effectiveness as many species are excellent indicators of habitat quality and management interventions. This manual gives an insight of for the tools and techniques which can be used for the monitoring the waterbirds in River Ganga.

Methods

How to Observe Waterbirds?

For observing waterbirds, you need some basic equipment including:

- Binoculars are essential. 8x30, 8x40, 10x40 and 10x50 are the most widely used by birdwatchers.
- If possible, a spotting scope (15-30x) mounted on a stable tripod.
- An identification guidebook
- Datasheets or Counting form
- Notebook and pencil
- A mechanical counter may help to count large flocks

How to identify waterbirds?

The basic tool for identifying waterbirds is a good field guide. First, try to identify the guild of the bird (e.g. dabbling ducks, diving ducks, plovers or stints) then, if possible the species. For proper identification carefully look at body size and shape, plumage and head patterns, as well as shape and color of the bill, legs, and tail. Be aware birds may appear larger in fog. Always look for direct comparisons with species you know.

Counting techniques

Ground count

Ground counts are the simplest and most common form of the census. The term refers to a count made from the ground, usually on foot. The site is covered systematically, usually by walking the same route on each visit and stopping every few hundred meters to scan with binoculars and/or a telescope to count the birds. When choosing a route (which is best done using a map in the first instance), thought should be given to light conditions (birds are easier to see with the light behind you), and to the risk of disturbing flocks of birds by your presence. It is important to use the best vantage points, and to divide the site up into areas that are visible from the chosen vantage points without overlap of areas counted and without missing any part of the site.Ganga basin is known for harboring globally threatened birds like Sarus crane. For the enumeration of this species vehicle transect is also a handy tool.

Picture: Point Count

Picture: Boat Survey

Boat survey

At many sites, especially large, remote ones, boat surveys may be the best way to count the waterbirds. Identifying and counting birds from a boat may, however, be difficult. Boats can cover large distances and give access to areas which would otherwise not be covered. They may also cause fewer disturbances that would be caused by surveyors on foot, although the opposite can also be true. Some of the difficulties with boat surveys include the low vantage point offered by small boats, the fact that they are unstable viewing platforms, often preventing the use of a telescope, and the fact that they are slow moving, so that any birds disturbed by the boat may be counted more than once. Boats also cannot be used in adverse weather.

Specialized methods

The methods described above will enable counters to successfully undertake counts (sometimes referred to as “core counts”). There are a number of additional, more specialized methods which are often used to complement or supplement these standard methods, and three of the most commonly used of these methods are described here.

Roost counts

Some species, for example, geese (Anser spp. and Branta spp.), waders (e.g. Haematopodidae, Recurvirostridae, Charadriidae and Scolopacidae), herons and egrets

(Ardeidae) and gulls and terns (Laridae), form large, concentrated roosts outside the breeding season. Other roost counts, for example of geese, should only be undertaken as part of a specially organized monitoring scheme, to ensure that birds at the roosts are not double-counted at their feeding sites. A preliminary scan with binoculars will locate the main concentrations of birds, and can be used to rapidly estimate the overall number of birds and proportions of different species, in case the birds are disturbed and fly away before detailed counting is finished. Accurate, species-by-species counts can then be made, ideally using a telescope and tally counter. Repeat counts are very useful under these exacting circumstances, and dividing the work between several observers helps prevent overload at big roosts. The sheer density of birds at the roost can cause difficulties, with birds at the back of flocks being particularly difficult to separate and identify. A solution to this problem that is often used is to count the birds as they fly in to roost. Counting birds in flight does present its own difficulties, however. It may be difficult to produce separate species totals for some large flocks of more than one species, and keeping track of rapidly moving flocks can be problematic. Finding a good observation position, using enough observers and getting the timing right are all factors that will improve the completeness and accuracy of high

Counts of colonially nesting species

Some species congregate at colonies during the breeding season, and closely coordinated counts at this time may be productive. Many species in the following families can be counted at their colonies: pelicans (Pelecanidae), cormorants (Phalacrocoracidae), herons and egrets (Ardeidae), storks (Ciconiidae), ibises and spoonbills (Threskiornithidae), flamingos (Phoenicopteridae), and gulls and terns (Laridae). Colonies in open terrain are relatively easy to count compared to colonies in trees, which are difficult to count accurately. It is extremely important to minimize disturbance of breeding birds and approaching too close. As with all monitoring, using standardized methods and counting the same sites in the same way each season are important crucial considerations.

Separate counts of different age and sex classes

Species with recognizably different adult and immature, male and female plumage classes can be separated according to these classes during counts. This is usually done as a part of detailed demographic studies. Sample age counts of many populations of geese and swans in the different part of the world and these extensive counts result in much-improved understanding of the productivity and population dynamics of these populations.

Introduction

Amphibians and reptiles are important components of ecosystems. Recently, there is an increasing awareness that, like for many other taxa, herpetological diversity is threatened, and the growing list of declining populations suggests a worldwide crisis (Blaustein *et al.*, 1994). Efforts to collect baseline data about occurrence, distribution, and status of populations are relatively well advanced for amphibians, but much less so for reptiles for which recent data suggest that turtles and crocodilians are as threatened as anurans (Gibbons *et al.,* 2000). This document is basic review of field techniques for sampling reptiles and amphibians.

1. Inventory and monitoring techniques:

This is a condensed review of the most common techniques used in inventories and monitoring of amphibians and reptiles whereby we focus on the possibilities, limitations and materials needed.

1.1 Standardising the sampling effort

In monitoring, the importance of having standardised sampling procedures cannot be overemphasised. The methods mentioned hereunder are suitable for both terrestrial and aquatic habitats, and may be an efficient way of standardizing monitoring surveys (*e.g.,* Brown 2001; Meik *et al.,* 2002). Next to recording biological data, the environmental conditions must also be scored and this again in a standardised way. Standardisation of sampling can be achieved through: (i) time-constrained searches; (ii) area-constrained searches; (iii) quadrat sampling; (iv) transect surveys.

2.1.1. Time-constrained searches

The premise behind this technique is to actively search for animals in a given area for a pre-defined amount of time. If additional information will be collected from the animals found (*e.g.*, body measurements or marking individuals), then the time invested in these activities should not be considered as part of the search. Time-constrained searches are mostly applied during terrestrial surveys, although they can also be used in aquatic habitats, particularly for amphibians. The main limitation of a time constrained search is the long periods that the survey participants must commit to it. Furthermore, it must consider that the results of time-constrained searches are highly influenced by environmental factors such as time of the day,

season, and weather (*e.g.,* it is well known that amphibian activity increases very much after rainfall). Another factor that will heavily influence time-constrained searches is the level of experience of the surveyors. Experts are likely to find more animals than inexperienced workers. It is vital to keep these factors in mind when designing a study. For an inventory it is advisable to repeat the sampling to include several days with different weather conditions and to always follow the same previously planned search routine (*e.g.,* if the first search included turning stones, then that should also be included when repeating searches).

2.1.2. Area-constrained searches

With area-constrained searches the search is focused on a certain area and not on an amount of time. Area-constrained searches will give information in terms of absence or presence of species, and potentially some data on life history of the species such as time of reproduction, activity patterns, and habitat use. The size of the area to be searched might vary but it will depend either on the habitat type (*e.g*., pond, creek, meadow, etc.) or on the focal species. The main limitation of this technique rests with the effect of environmental conditions, the experience of the workers, and the planned search routine. As with time-constrained searches, the searches should be done during several days with different weather conditions or even different seasons to maximize the chance of encountering all species present in the area.

2.1.3. Quadrat sampling

In this technique, sampling arrays in a study area must be randomly distributed and the absence or presence of animals in these arrays verified. The sampling areas are usually squares (quadrats) that are thoroughly searched (Jaeger & Inger, 1994). The main drawback of quadrat sampling is that the setup can be very timeconsuming. Within quadrat sampling, we can differentiate point sampling where small squares are used, and broad sampling where larger quadrats are used (Kok & Kalamandeen, 2008). Point sampling is preferred when studying single species in which the individuals are relatively small and densely distributed, while broad sampling is applied to species that are widely dispersed, large bodied or both, as well as for multispecies assemblages. In both cases, all quadrats need to be of the same size within each study area. A modification of this technique is called ‘patch sampling’, in which the sampling arrays are normally specific microhabitats (*e.g.,* logs, bushes, etc.). Patch sampling is applied when looking for specific target species, which we know or suspect that are confined to specific microhabitats within a larger habitat (Jaeger, 1994a). For both techniques some pre-requisites have to be met.

For quadrat sampling:

- Animals may not leave the quadrat before being observed.
- The quadrats are randomly distributed.

For patch sampling:

- Each patch must be defined precisely and in an operational way.
- All patches must be equally locatable by the observer without any bias.
- Animals may not leave the patch before being observed.

If these criteria are met, then quadrats and patches can be distributed randomly within the study area. Each of them then represents an independent sample, allowing statistical analysis of the obtained data if at least 25 to 30 quadrats were scored (Jaeger & Inger, 1994). Quadrat sampling has proved to be particularly useful in forests when searching for ground-dwelling amphibians and reptiles (Rodda & Dean-Braley, 2002). For best results in this methodology of quadrat (or patch) sampling, it will be important to apply the most appropriate searching technique within each of the quadrats (*e.g*., using a rakes over leaf litter).

2.1.4. Transect surveys

A linear transect is established and the whole narrow strip (and nearby areas) is searched for animals. This is usually utilised for surveying herpetofauna across environmental gradients but can also be used within a single habitat (Jaeger, 1994a). However, for homogeneous study areas, quadrat sampling is recommended. If the design is properly randomized this method will provide a good representation of the occurring fauna over all habitat types. Depending on how the transects are set regarding the gradient, different information will be obtained. If transects are set in parallel to the gradient studied, then these surveys may be used to compare species across habitats. If on the other hands, transects are set perpendicularly to a gradient (*e.g*., along a river), then one will be able to study changes in parameters of a given species along the gradient. The most common scale used in transect surveys is at the habitat level, but it is possible to work on a larger scale (ecosystem or landscape) by using, for example, aerial surveys across a large transect (Mour.o *et al*., 2000). Ideally, transect surveys have to meet the following assumptions:

- Specimens are randomly distributed throughout the transects.
- All the specimens in the transect will be observed.
- Animals will not be counted twice within a transect and among transects.

When preparing transect surveys it is important to consider that some species will not meet all the method's assumptions. For example, cryptic species will not be observed or will flee from the observer without any notice, or many species do not have a random distribution, as they are associated to specific microhabitats.

2.2. Sampling Techniques

For selecting the most suitable sampling technique, it is necessary to evaluate :

- The objective of the study.
- The conspicuousness of the species of interest (their activity and habitat).
- The cost, time, and resources needed.

By and large, the methods that are more time- and resource-intensive will yield most information, and will allow more powerful statistical analyses. However, depending on the goal of the study, such intensive methods might provide data that are not needed (*e.g.,* obtain detailed ecological data when presence or absence of species would suffice). Furthermore, it is also more productive to use a combination of techniques instead of applying a single one, but again this will require more resources. Therefore, one must strike a balance between available resources for research and desired results before starting fieldwork. The most common techniques used for sampling reptiles and amphibians can be divided into active and passive sampling, each with a number of specific techniques.

2.3. Active sampling

2.3.1. Visual encounter surveys (VES)

VES is by definition a time-constrained method in which observers sample for species richness and abundance along a survey path (Crump & Scott, 1994). The time spent in the field and the numbers of observers are taken into account. This technique is appropriate for both inventory and monitoring. VES might be particularly useful for detecting rare species that seldom fall into traps, and thus by using VES in combination with a passive sampling technique, it is potentially possible to obtain the complete species composition of the sampled area. Nevertheless, the efficiency of VES will vary much depending on the type of habitat (*e.g*. low vs. high vegetation) and the species biology (*e.g.* fossorial vs. arboreal). As a matter of fact, visual encounter surveys have a number of assumptions that in many occasions cannot be fully met :

- Each individual of every species must have the same probability of being encountered. This will not be met for example in species with a large sexual dimorphism were one of the sexes is much more visible than the other;
- Each individual is only recorded once during the survey. For this the use of individual marking may be the solution, but it implies a higher time investment;
- Each observer doing the survey must have similar experience and be able to potentially obtain the same results. The best approach to this problem is by training the workers in advance to ensure a similar level of experience. Road cruising and aerial surveys could be cited as visual encounter surveys, although these are done at a different scale and have specific characteristics.
- In the case of road cruising, a road is used as a survey transect that is methodically driven through looking for both alive and roadkill specimens (Andrews, 2008). Aerial surveys are mostly used for estimating population size and distributions of large-bodied reptiles such as crocodilians or sea turtles (Glaudas, 2008).

2.3.2. Dipnetting and kick sampling

We refer to dipnetting when a dipnet is swept through an aquatic habitat to capture herpetofauna. When the dipnetting process is semi-standardised – thenumber of sweeps is recorded and compared among habitats one may call it sweep sampling (Dodd, 2003). Sweep sampling is used for sampling herpetofauna in small aquatic habitats (treeholes, springs, puddles, and ponds) where it is more efficient. However, sweep sampling may be used in larger aquatic habitats such as lakes as well with the aid of seines and nets. The main targets of this technique are amphibian larvae. It is important to consider that specific differences in animal positioning in the water column may result in differences in the ability to catch different species. Also not all species can be caught with the same net, so the type of net and its mesh size must be carefully selected depending on the ecology and size of the targeted species. Moreover, dipnetting should be scheduled in the season when the species are most likely to be found in the water. When these factors are taken into consideration sweep sampling may be a very effective sampling method that allows for comparisons among aquatic habitats that are somewhat homogeneous.

Kick sampling is a technique that is especially fit for aquatic habitats of small to intermediate size and with fast flowing current. It is predominantly used when looking for stream dwelling

amphibians. It consists of lifting and removing all loose substrate from a stream bottom, kicking loose pebbles or even hand raking everything into a net. Typically, the most common nets used are those with a Dframe whose flat side may rest on the bottom of the stream. The most efficient way to kick-sample is with two-person teams, where one worker loosens the woody debris, rocks and other substrate, while the other holds the net in place. It is very important when sampling for herpetofauna to check the nets very often (every 5 minutes or less) to decrease animal stress and mortality. As before, mesh size has to be considered based on the target species size. The smaller the mesh size, the more species will be captured, but small mesh nets tend to clog up faster with debris, and thus require more frequent maintenance to maintain efficiency. The main limitations of kick sampling are that it is very labour intensive and that it can cause habitat disruption. For the latter reason, it is very important to redeposit the habitat items (*e.g.* large stones, wood debris, etc.) that had been moved.

2.3.3. Stovepipe sampling

Stovepipe sampling is a quantitative method in which aquatic animals are trapped within an enclosure and later removed from it with a net (Shaffer *et al.*, 1994). The enclosures or samplers are typically pipe-like (one may use air conditioning ducts, culverts, stove pipes, and PVC pipes) (*e.g.,* Alford, 1986 or Skelly, 1996) or a rectangular box (*e.g*., Harris *et al.*, 1988). These samplers are placed in the water, firmly set against the substrate, but with enough care as to not disturb the environment and cause the animals to flee. Once the sampler is in place, a net is swept within the enclosure to collect the animals. This technique is especially suitable for obtaining quantitative estimates of larval densities that can be used to estimate population size. Samplers should be placed randomly across the habitat, and their dimensions and the water depth recorded to obtain values of captured animals per volume. The best habitats to apply stovepipe sampling are shallow waters with sandy or mucky substrates, which allow to easily install the samplers. In habitats with water vegetation, pipe enclosures are easier to install than rectangular ones. This technique can be time intensive to use, so in case of large habitats or if we only want to determine the presence of a particular species, other methods such as dipnetting will be more useful.

2.3.4. Egg mass and nest counts

This is a method that can be used during breeding periods to monitor the reproductive activity in reptile and amphibian populations. In amphibians, egg masses are counted around a pond

perimeter or within the pond and it is particularly useful for explosive breeders and those that reproduce in communal aggregations. For identification purposes it is recommended to photograph the egg masses or at least use detailed language to describe it. Mitchell (2000) recommends making the following observations:

- Is the mass globular or round?
- Are the eggs clumped, separated or on a string?
- What colour and shape are the embryos?
- Is jelly surrounding the eggs firm or loose?
- Is there a film on the surface of the mass?
- To what type of vegetation is the mass attached?

In the case of reptile egg nest counts, this technique is most useful for turtles and crocodilians. Normally a relatively large area must be checked and there is need of having some previous knowledge of nesting grounds, and sometimes the recognition of tracks can be very useful, as well as the leftover from predation over the nests or the remnants materials after the babies hatch (*e.g.*, broken egg shells). Egg mass and nests counts is a relatively simple and powerful method for determining the presence of species, and especially in the case of species that lay a single clutch per year it can be a reliable indicator of population size. This technique is nevertheless useless for amphibians that lay eggs in the land and for most squamata reptiles. Finally, it is important to consider that the lack of egg masses or nests cannot rule out the possibility of a species being present, but not reproducing.

2.3.5. Auditory surveys

Auditory surveys are very useful for estimating species richness of anurans. Male anurans in particular tend to be fairly conspicuous during breeding season when the use their mating calls for attracting females. These calls are species specific, so during the breeding season listening stations can be randomly selected along the breeding site to identify species presence and their relative abundance. This technique has the advantage of easily covering rather large areas while being hardly non-invasive.

Not all anurans are equally easy to detect, but with some training even nonexpert workers can obtain good results. In inventory, regular auditory surveys are very helpful for determining species composition, but there are some limits when it comes to monitoring changes in a population because there is always a bias towards only observing declines in calling activity and it is difficult to evaluate if these are due to natural fluctuations. If the aim is monitoring,

acoustical surveys should always be coupled with other sampling techniques. In the chapter on bioacoustics more information can be found.

2.3.6. Basking surveys and basking traps

Sampling techniques based on the animal's basking activity are applied in aquatic habitats, especially rivers were the observer can advance in parallel to the river bank while scanning basking sites with binoculars. The studied animals are normally turtles (Buhlmann & Vaughan, 1991; Lindeman, 1998), although it has also been applied on water snakes (Mills *et al*., 1995). Apart from species presence, basking surveys can also give information on sex ratios and juvenile recruitment, but when further information is needed, this technique is to be complemented with basking traps. These are wire traps that are attached to the underside of the basking log so when the animal instinctively jumps into the water, dives to the bottom of the trap giving the observer time to retrieve it. It is important to remember that basking traps must allow the animal to ultimately climb out of the trap if they fall in and the researcher is not present. The effectiveness of the basking surveys will depend on the amount of basking surface available, the time of the day or season when it is done and the animal's basking behaviour. The main limitation of this technique is that it depends on amount of basking surface available. If there are no basking sites, then no animals are observed, but it does not mean that the species is absent. For using basking traps it is absolutely necessary to identify first favourite basking sites, so a basking survey will always precede the setup of basking traps. In monitoring initiatives basking surveys and basking traps should always be made in conjunction with mark-recapture studies.

2.4. Passive sampling

2.4.1. Artificial cover

Many reptiles and amphibians use covers in the wild for hiding. Logs, rocks and even human debris provide refuge to many species, which implies that sampling these covers many times is an effective method. The problem with these "natural" covers is that quantifying their effectiveness is difficult. By using artificial coverboards we can standardize the sampling effort maintaining the natural habitat and limit biases. The materials most commonly used for coverboards are solid wood boards, plywood boards, corrugated metal strips, tarpaper and horticultural plastic sheeting. These coverboards are set in array designs as linear transects, rectangular grids or webs, depending on the species and/or habitat sampled. Artificial coverboards have been used to sample many species of reptiles and amphibians (Parmelee &

Fitch, 1995; Sutton *et al*., 1999; Houze & Chandler, 2002; Ryan *et al*., 2002; Smith *et al*., 2006). An additional benefit when using coverboards is that as they do not restrict movement, it does not require continuous surveillance as for example pitfall traps. Their maintenance is also easy and inexpensive when compared to pitfall traps. In studies where coverboards and pitfall trap arrays have been used, pitfall always captured more species and more individuals (Sutton *et al.*, 1999; Ryan *et al*., 2002), although coverboards detect species that are not found in pitfalls. In this sense this technique has proved to be particularly useful for small secretive snake species (Fitch, 1992). When checking coverboards it is advisable to use tool such as snake hooks to avoid accidental bites. It is also advisable to flip the coverboards always towards the researcher to avoid the animals to escape. Finally, when sampling the coverboards it is also advisable to record environmental data such as the weather conditions, time of the day or the temperature. Sampling encompassing as many environmental conditions as possible will always yield better results.

2.4.2. Polyvinyl chloride (PVC) pipe surveys

PVC pipes are an easy and inexpensive technique for sampling hylid tree frogs. These PVC pipes can be placed in the ground or mounted on trees following a grid or transect setup. The ground-placed PVC pipes can be used nearby the breeding areas of the hylids or as a complement to pitfall traps and drift fences, which are normally easy to avoid for the tree frogs. The tree-mounted PVC pipes on the other hand are suitable for sampling the tree frogs even outside their breeding season (Dodd, 2003). For ground-placed pipes a good length is 1 m vertical pipe with around 60 cm sticking out of the surface, while tree-mounted pipes can be of around 60 cm with the bottom part set at a height of 2-4 m.

These pipes should have the bottom sealed with a cap to retain some water, but holes should be made in the pipe at about 15 cm to allow draining the excess of water. A good average diameter for the pipes in both cases is about 2-5 cm. Nevertheless, tree frogs can be of many different sizes, so it might be necessary to try out pipes with different sizes and diameters until finding the most successful design for a given species. An important benefit of PVC pipes is that it causes no mortality on the sampled animals, so the frequency and timing of the checks can be very flexible. This allows accommodating this technique easily with other activities and also makes it suitable for using in remote field sites. The main limitation it has is that it is very specific (only for tree frogs) and that PVC pipes are rather conspicuous, so they can be subject of theft or unwanted manipulation. This technique is most useful for detecting presence/absence of species, and even for determining timing and dispersal from

breeding grounds. On the other hand it is very tricky for comparing between sites because its results will depend very much on species assemblage and on the availability of other natural hiding sites. If the aim is monitoring through time in a same site, PVC pipes in conjunction with marking individuals can give much information.

2.4.3. Leaf-litterbag surveys

Leaf-litterbag surveys are specific for salamanders, which can be difficult to monitor due to their cryptic and fossorial nature. Litterbags have been commonly used for many years to estimate leaf litter breakdown in streams (*e.g.*, Peterson & Cummins, 1974), but it has been adapted for sampling stream-dwelling salamanders (Pauley & Little, 1998). This technique was successfully applied in the Great Smokey Mountains National Park to inventory various streams (Waldron *et al*., 2003). Their basic design consists of a square (50-90 cm per side, with 70 cm x 70 cm being the optimal size) piece of plastic netting with 1.9 cm mesh. Small rocks are placed on the netting in the field and covered with leaves before the corners are brought together and bound with cable ties to form the litterbag. Finished bags are placed in the stream at regular intervals and after an acclimation period of a couple of weeks, each bag is checked by placing a dip net underneath and lifting the bag into a bucket of water. Then, to extract the salamanders from the bag, dip the bag repeatedly in the bucket and then pour the water through the dip net. The salamanders are then processed and the bags are placed back into the stream. Although this technique has proven to be successful for detecting the presence of salamander species, it is not capable of indexing populations sizes, so it cannot be applied on its own in monitoring programs.

2.4.4. Aquatic and terrestrial funnel trapping

Funnel trapping is a standard method for trapping many groups of animals including reptiles and amphibians. The principle behind these traps is pretty simple: animals are directed through a small opening in the trap via a funnel or ramp, and once inside, are unable to find their way out. This is a technique especially useful for capturing rare cryptic species and has the advantage of being suitable for standardizing. In addition as traps are used during a lapse of time, this technique is also less sensitive to biases resulting from temporal variations. On the other hand funnel trapping requires a substantial investment of time and equipment. The traps themselves can be expensive, and should be checked often to avoid mortality of the trapped animals. When applying funnel traps in inventories, the effort should focus on habitats and times when the target animals are more likely to be active, and the more different

habitats sampled the more species we will likely detect. Nevertheless, funnel traps have generally a low capture rate, so for successful inventories, a high intensity sampling is recommended (several hundred trap-nights spread across the season). Funnel traps are also very useful in long term monitoring programs as the trapping scheme can be easily replicated allowing comparisons. For this, traps can either be set in systematic or random arrays. Based on the capture rates detected we will be able to infer population status, but always with some reserves, as capture rates will depend not only in population size, but also in level of activity and the propensity of the species to enter and remain in the traps. The ideal situation is when traps are complemented by mark-recapture data. Funnel traps can be used in aquatic and terrestrial habitats, and can be of different sizes, materials and shapes. The use of one or other will normally depend on the target species:

- **Small aquatic funnel traps**: These can be either cylindrical or rectangular and are normally used for trapping water snakes and aquatic amphibians. The traps that are commercially available are designed for capturing crawfish or eels, but these can also be used for amphibians. They are typically double ended and built of steel hardware cloth, plastic or nylon mesh. The plastic traps are normally the most suitable for trapping the smaller species. As an alternative, small and inexpensive traps can be made by inverting the top of a plastic soda bottle and anchoring it to the substrate with a stake (Willson & Dorcas, 2003).
- **Hoop-nets**: These are large funnel traps used primarily for trapping highly aquatic carnivorous turtles, although it is potentially useful for trapping any aquatic turtle. These traps are also commercially available in different sizes and made of twine or mesh. In their setting, the traps normally have a part above the surface allowing the captured turtles access to air. Normally hoop nets are baited to increase success and should be checked at least daily. In occasions hoop nets can also capture large aquatic salamanders and large snakes.
- **Interruption traps and fake nets**: These traps are suitable for complementing the hoop nets. In this case the trap is unbaited, but uses nets or natural channels to draw the turtle towards the funnel. Essentially they work like drift fences but on the water. The design of the trap can include unbaited hoop-nets, swing door traps or pressure plate traps at the end of the channels of nets. As with the hoop-nets, although these traps are mainly for turtles, they can capture other species such as large amphibians or large snakes (Vogt, 1980).

- **Terrestrial funnel trapping**: Terrestrial funnel traps are typically used in conjunction with pitfall traps along drift fences. The design of the trap can be very variable, although the most common variation consists of a wire hardware cloth cylinder with inverted hardware cloth funnels pinned into each side (Fitch, 1987). It is advisable to set the traps in the shade or cover them with a board to make them more attractive and to protect the captured animals from the rain and the heat. In the case of amphibians it is also advisable to use some kind method to moisture the inside of the trap (*e.g.*, a moist sponge). Terrestrial funnel traps can also be constructed of wood boxes, which makes their building more complex and time-consuming, but in different studies have proved to capture almost any snake, reptile or amphibian possible (*e.g.*, Burgdorf *et al.*, 2005; Enge, 2001; Greenberg *et al.*,1994).

2.4.5. Terrestrial drift fences and pitfall traps

Drift fences have proven to be effective for sampling most amphibians and squamata reptiles (Nelson & Gibbons, 1972; Semlitsch *et al.*, 1981; Hanlin *et al.*, 2000; Enge, 2001; Russell *et al.*, 2002; Ryan *et al.*, 2002, Todd *et al.*, 2008). The basic design of a drift fence is a straight fence buried slightly below ground, and standing up to 50 cm high. Pitfall traps are then buried at floor level and placed at a certain interval alongside the fence. The spatial arrangement of the fence can vary, and we can separate drift fence arrays into:

Straight-line drift fences: These can be set up in X or Y-shaped arrays and are normally used for sampling upland habitat (Corn, 1994).

Continuous or partial drift fences: This setting is commonly used to circle partially or completely wetlands (Dodd & Scott, 1994). The capture rates and effectiveness of this technique may differ very much between sites, but it is clear that this technique is particularily useful for determining species richness and relative abundance (see Ryan *et al.*, 2002 for comparisons with coverboards and time-constrained visual surveys).

The main limitations are as follows:

- Expensive and hard to set up. After installing, the traps should be visited at least once in a day.
- Capture biases. Some species may show trap avoidance or even attraction towards the pitfall traps.

- Many species such as large snakes or tree frogs can escape from the pitfall traps. This can be somewhat avoided with putting plastic collars on top of the pitfall traps or using double-pit systems. Species associated to certain microhabitats might not be sampled. The best way to improve the success of drift fence arrays is to combine pitfall traps with funnel traps. This technique is normally used on long term monitoring programmes due to the relatively high amount of time and funding needed to install them.

3. Capturing and handling animals

When sampling animals they should be handled in a way that allows further study (vouchering, photographing, marking, etc.). Handling is generally done by hand, but several tools and utensils can ease the task and increase the safety of both the sampler and the specimen.

3.1. Snakes

Prior to identification, all snakes should be considered potentially venomous. When identified as venomous, ONLY EXPERIENCED AND TRAINED PROFESSIONALS SHOULD EVER ATTEMPT TO CAPTURE AND HANDLE THEM.

The most common tools used for capturing snakes are hooks and tongs that are used to immobilize the snake and keep it at a safe distance from the researcher. The usual procedure for manipulating a snake is using the hook or tong for lifting up the animal gently from the mid-front body while keeping hold of the snake's tail to avoid it from turning around.

For hand-catching snakes, we should set it in an open area and press its head gently, but firmly against the floor, using for example the bottom of the hook, so we can safely manipulate it. We can secure the head between the thumb and fingers of one hand, and use the other hand to sustain the rest of the body weight to make sure the snake does not suffer spinal injuries. Although giant snakes (boas and pythons) are not poisonous we should never underrate their strength. They should never be handled by only one person and special care must always be paid to their heads. These animals need to bite in order to strangle and their bites can easily infect due to the bacteria in their mouth. For smaller harmless and fast moving colubrids, hooks and tongs might not be appropriate and collecting directly by hand with thick protection gloves is recommended.

Hooks can easily be handcrafted, but tongs are more difficult to manufacture and are normally purchased from supply companies. Currently both hooks and tongs from different brands are readily available through the Internet. They should be made of a light but resistant materials such as anodized aluminium or titanium. The size of the hook and tongs will depend on the size of snake we target. The handles of both tongs and hooks should be made of a material that will not slip during the manipulations, such as rubber. Finally the material that will be in contact with the snake should minimize the chance of injuring the snake while manipulating it (*e.g.*, rubber coated).

3.2. Lizards

Lizards on average can be quite difficult to capture by hand due to their size and fast movements, so to assist on their capture we can use a small noose. The noose can be built with a long, slender pole such as a bamboo stick or a telescopic fishing pole where a thread of dental floss or fishing line can be attached. It is common lizard behaviour to flee upon sensing something approaching and then freeze shortly, and it is then when the noose can be placed over the head to trap the animal from a certain distance (see Marcellini & Jenssen, 1991). In the case of large lizards, caution must be taken when handling as they can cause injuries with their claws, and deliver powerful bites that can easily become infected. It is recommended to manipulate these animals wearing heavy-duty gloves to prevent any possible wounds. It is very important to avoid capturing lizards by the tail as it will break off in many occasions.

3.3. Aquatic turtles and tortoises

Aquatic turtles can sometime be captured by hand and with the aid of a dip net, although the usual way of capturing turtles is using traps (see survey methods). In the case of turtles or tortoises they should always be handled with care as they can deliver powerful bites, but this is easily avoided by keeping your hands away from their head. Normally turtles can easily be held at mid- or back-body, although additional attention should also be paid for some species' claws that can be elongated and inflict deep wounds. As with large lizards the use of thick gloves to manipulate the animals is also recommended.

3.4. Crocodilians

Due to their size and dangerous bites, crocodilians should exclusively be handled by experts. Normally their capture is done by several people and with the aid of a noose. While small and

young animals can be grabbed from behind the head with one hand, using the other hand to support their weight (as you would do with a large lizard), larger animals have to be handled by several people. It is important to make sure that the jaws are closed, for example by wrapping duct tape around them, before doing any measuring, and extreme caution must be paid to the tail which can deliver powerful strokes. It is highly advisable to cover the animals' eyes to reduce their stress.

3.5. Amphibians (frogs, toads, newts, salamanders and caecilians)

Aquatic amphibians can be captured by hand and with the aid of a dip net before they jump into the water or while floating in shallow waters. Most amphibians are nocturnal, so a flashlight can also be used to temporarily blind them and get close enough to them. In the case of terrestrial amphibians the challenge is locating them, as on average capturing them by hand is not difficult. Nevertheless we should have in mind that all amphibians have some degree of toxicity in their skins. Cutaneous glands are a shared character of all adult amphibians and they are normally the main source of biological active compounds found in the amphibians skin. The level of toxicity depends on the exact components of these substances and can range from noxious to highly toxic depending on the animals. The highest toxicity is due to the presence of alkaloids that in most cases derive from the arthropods the animals eat in the wild. Alkaloids have been found in some salamanders, but especially in Dendrobatidae and Mantellidae (Daly, 1998). The secretion of these compounds will be increased when the animals are stressed due to handling so the use of latex gloves or an inside out Ziploc bag is recommended to avoid direct contact with the skin. If none of these are available, and we must necessarily have direct contact with the animal, hands should always be thoroughly washed after manipulating them, making sure we avoid contact with our eyes or mouth. In the same way, any surface that has been in contact with the animals should be thoroughly rinsed and cleaned with water.

For safely handling frogs and toads, they should be held between the fingers and thumbs around the waist of the animal. For some specific measurements or for photographing the frogs should be grabbed from one of the front legs between the thumb and index finger while sitting on top of the hand. The grab should be firm enough to avoid the animal from escaping using their strong back legs, but with much care to avoid any damage to the front limbs. In the case of salamanders and newts, we should hold them in the entire hand gently restraining the animal between the thumbs and fingers just behind the head, in a similar way as it is done with medium and small-sized lizards.

Finally, it is important to consider that when handling different amphibian specimens in the field, a researcher can involuntary become a vector for transmitting pathogens such as chytrid fungi. The chytrid fungus *Batracochytridium dendrobatidis* is behind the disappearance of entire populations of amphibians around the world, so if your are going to handle amphibians in the wild, there are a number of rules you should strictly respect to avoid the transmission of chytrid fungi between populations or sites:

- Never move individuals of adult amphibians, larvae or egg between distinct places even if they are very close since this could contribute to the dispersion of the pathogens;
- Never introduce animals, plants or any other organism in the environment, because, besides interfering with native species, they may carry pathogens. We know that fish can transmit viruses that affect amphibians, and in many countries the native amphibians are infected by introduced amphibian species that carry the chytrid fungus. If you detect introduced (allochtonous) organisms in your area, get in contact with an expert;
- Avoid accidentally transporting the pathogens yourself. The chytrid fungus does not have a stage that is resistant to desiccation but it can survive in whatever type of organic material that maintains humidity. As such, after a trip to the field wash well at the site all the objects that have been in touch with the environment (*e.g.*, boots and sample nets). After submerging them in bleach (a bath of 30 seconds is sufficient if you use domestic bleach with at least 4% sodium hypochlorite) or in other suitable disinfectants put them out in the sun for as long as possible;
- If you do not want to use bleach to clean your field material, you can use commercial products specifically sold in veterinary stores. Some suitable commercial products are: Halamid. (www.alpharmaanimalhealth.co.uk) and Virkon. (www.antechh.com);
- If you hold amphibians use disposable gloves or if it is necessary to keep them for some time use disposable containers or ones that have been previously sterilised. Do not put them in touch with specimens from other areas if you are going to return them to the natural environment. Remember that you must sterilise all equipment before using it;
- Inform when possible about the problem of emerging diseases in amphibians and how it is possible to avoid contributing to its spread.

4. Transporting and housing captured animals

If the captured animals must be transported to the lab and housed for some time it is necessary to use appropriate containers. In the case of amphibians it is most important to keep them in moist substrate in containers or sealed plastic bags. It is a good practice to include some leaves or leafy branches to prevent squashing and maintain humidity. A moist paper towel or standing water in the container usually is effective depending on the needs of the species in question. For tadpoles, plastic containers filled in with water from the capture site can be used, and these containers should be transported in lightly chilled coolers to keep the tadpoles with a relatively low metabolic rate.

Small containers with ventilation are useful for holding small snakes, small turtles, and most lizards. Cloth bags of all sizes, including pillow-cases, are useful for temporarily holding even the largest lizards, turtles, snakes, and small crocodilians. One must be careful not to allow the animals to suffocate or drown while transporting them, and avoid placing them in direct sunlight where any container can rapidly overheat and the animals inside die. Once in the lab, the setting prepared for short term housing the animals can be very simple. One must make sure that the temperature is suitable for the animals, that natural photoperiods are respected and that the containers are clean and have sufficient water and food.

5. Collecting information from captured animals

5.1. Measurements

All amphibians, squamata reptiles (lizards and snakes) and crocodilians the standardized measure used is the snout-vent length (SVL) that is defined as the distance between the tip of the head and the end of the cloaca. In addition, the tail length can also be recorded to have the total length of the animal, but salamanders and squamata reptiles have the ability to loose their tails as a defensive mechanism upon being attacked by a predator. Together with the measurements of the body length, the typical measurement is weight. Most herpetofauna can be weighted with either a spring scale or an electronic scale, but for larger species (giant snakes, crocodilians, large turtles) a truck scale will be necessary. Due to the ectotherm nature of reptiles and amphibians, in many occasions, it will also be of interest to obtain the cloacal temperature of the animals. Ambient temperature can be used as an approximation if it is not possible to measure body temperature, but it must be remembered that there can be significant differences between both measurements due to fluctuations that the animals metabolism can produce in their body temperature. The body temperature of the animals will affect their activity, so this information can be relevant for comparing between sampling

periods in a monitoring activity. For measuring the body temperature we can use cloacal thermometers or digital thermometers with a probe. Take into consideration that, especially for smaller specimens, contact with our hands will affect their body temperature, so the measuring of temperature should be done immediately upon capturing the animal.

After collecting the animals it can sometimes be necessary to preserve them as vouchers. The preservation of specimens is a key element for taxonomic identification and when accompanied by properly compiled field notes, it becomes an excellent resource for scientific research in many branches of biology. For example, historical data from museum specimens can allow researchers to detect and assess changes in biodiversity in an area over time. For the preparation of vouchers it will be necessary to kill the animals, although in some cases it is possible to use animals that are already dead due to traps or road mortality. We should collect the minimum number of specimens possible depending on the aims of our study. Although it can depend on how common the animal in question is, it would be advisable to preserve around 20-30 animals for scientific studies and a minimum of 4 for voucher specimens (Graeter *et al.*, 2008). It is mandatory to follow any institutional guidelines that may apply or to request the necessary permits. The procedure to euthanize the sampled animals should be humane and should preserve the condition of the animal. The most preferred techniques for killing reptiles and amphibians are by injecting or submerging the animals in lethal doses of one of the following:

- Sodium pentobarbital
- Hydrous chlorobutanol
- Tricaine methenesulfonate
- Cloretone
- Ethanol
- Other anesthetics

In the case of amphibians, due to their permeable skin, immersion in anaesthetic solutions is the most frequent way of humanely killing them. The most common products used are chlorobuthanol and tricaine methanesulfonate, also called MS- 222 (Andreone *et al*., 2008). The minimum concentration should be 250 mg/l (concentrations >500 mg/l must be buffered with an equal weight of sodium bicarbonate as it is an acidic product).In the case of reptiles, sodium pentobarbital has traditionally been used injected intravenously, intra-abdominally or intrapleuropitoneally (Cooper *et al*., 1989), but recently the use of MS 222 has also been

recommended through intracoleomic injections of 250 to 500 mg/kg at 1% solution (Conroy *et al.,* 2009). The fixation of the specimens should only begin once we are sure that the animals are dead. As chemical fixation affects the proteins in the tissue of the animals, we should attempt to fix them in positions that preserve their morphology and that allows for the observation of key identification characters. The fixation in 10% formalin (obtained by diluting 40% formol) allows a better preservation of morphology so it is ideal for the animals that will be used for formal taxonomic description or for exhibit. Formalin is carcinogenic, flammable and dangerous if fumes are inhaled, so the appropriate cautions must be taken when working with it. In addition it will not allow to use the specimens for posterior DNA analysis so it is advisable to collect tissue samples before fixing the entire specimen, and fix these in pure ethanol. In case formalin is not available, 70% ethyl alcohol can be used, but other alcohols are not recommended (McDiarmid, 1994).

Once the specimen is fixed, it is extremely important to attach each specimen with data such as the field number and any information recorded from the field (GPS coordinates, time, habitat, initial identification, collector, sampling method or weather conditions). It is advisable to use acronyms in the field number referring to the collector, followed by a progressive number and keep the same structure within sampling efforts. This should be printed in hard paper resistant to ethanol and formalin; either hand-written or printed with water resistant ink as there is a risk of loosing the information during transport or long-term storage.

Dependent of the aim of the study it could only be necessary to take a tissue sample or biopsy from the captured animals instead of preserving the whole specimen. Blood samples are the most common procedures as when it is correctly done it may be less invasive than taking other tissues. In the case of DNA analysis rather small amounts of blood will be necessary, although the amount will be larger for physiological studies. Turtle blood can be obtained from a femoral or jugular vein, a carotid artery, the retrorbital space or the paired cervical sinuses (Dessauer, 1970). In medium and large sized lizards blood is typically collected from orbital sinuses (*e.g.*, Haenel *et al*., 2003), and in crocodilians blood is normally taken from internal jugular or caudal veins. In the case of amphibians, only the larger species can endure blood sampling and this can be done through the midline abdominal vein. Finally for most relatively large reptiles and amphibians heart puncture can also be a viable way to extract blood although this can cause mortality if done by inexperienced workers. In the case of smaller animals, heart puncture will be the only way to take blood samples and will necessarily be fatal. The blood samples can be collected through heparinized capillary tubes.

Alternative tissue samples that can be collected in reptiles and amphibians are tail clips from salamanders, lizards, turtles or snakes. Toe clips may be used as well in salamanders, frogs and lizards, while clipping scutes of the tail of crocodilians and ventral scales from snakes are also common practice. These sampling techniques have the additional benefit of potentially being very useful to researchers who need to mark animals for individual identification. Finally for DNA studies there is the possibility of using other non traditional sources of tissue which are not aggressive but can later prove difficult to analyze due to the low molecular weight and concentration of DNA in the samples. The most relevant of these sources in amphibians and reptiles are feces, although orifice swabs and shell or scale remnants can also be useful (Poschadel & Moller, 2004). For methods to better preserve tissues for future DNA analyses, we refer to the chapter of Gemeinholzer *et al*. (this volume) on organizing specimen and tissue preservation techniques in the field for subsequent molecular analyses.

5.2. Photo-vouchering

Photo-vouchering entails using photographs to document the occurrence of encountered wildlife. This is particularly useful in herpetology as it is very possible to make photographs of the animals accenting the key features that allow for a doubtless identification. These photo-vouchers, if correctly complemented with additional information will provide long-term evidence that those species exist or existed in a given geographical location. A literature record complemented with a photograph will make the report reliable without the shadow of a doubt. In addition photo-vouchers can be the alternative to traditional vouchers in the case of rare, threatened and endangered species or the alternative to the records of animals difficult to capture, such as basking water turtles. In the cases when the preparation of vouchers specimens is

absolutely unavoidable, photographs of the living animal will also be of much help as after fixation specimens tend to lose their colours and even some patterns. The ideal situation of documenting the occurrence of a certain species is having the voucher specimen for detailed analysis complemented with photographs of the specimen before fixating. Currently the use of digital cameras has made photographing cheaper. It is possible to quickly review the photographs taken and make as many pictures as necessary, although we should remember that digital files can also become corrupted and the information lost. Some recommendations for preparing photovouchers are:

- If the photographs are going to eventually be deposited in a natural history museum or other repositories we should obtain information on the format, size and resolution needed;
- Include some kind of scale in the photograph to have information on the size of the animal photographed;
- Make the photographs of the animals as soon as possible after capturing, as especially some amphibians tend to change colours and patterns after being captured;
- If the animals are very active, it can be useful to lightly chill them in a refrigerator, but never in the freezer. The amount of time should never be over a few minutes depending on the size, and if the animals are later going to be released back to the wild, first make sure that it has returned to normal temperature before doing so.

6. Field notes and data collection

Most serious shortfalls in gathering and managing descriptive data on amphibians and reptiles can be avoided through planning and preparation prior to collecting data. The list below compiled by Greene (2008) includes common issues and problems that need to be addressed when implementing an inventory or monitoring program:

Research and study goals and the specific data to be gathered must be clear to all parties involved (*e.g*., funding agency representatives, researchers, and technicians);

Data must be gathered in an organized, consistent manner. Design a datasheet that is objective and simple to use, and which includes all relevant information in sufficient detail. If funds and expertise allow it, invest in personal digital assistants (PDAs) or electronic laboratory notebooks which can be programmed with customized forms for direct data entry in the field (this can help minimize data entry and data transfer errors); All personnel involved must be trained to gather data in the same manner. Attention to detail and consistency are paramount. Handwriting must be legible; Store data routinely in one place until the data can be entered into a database. Keep electronic backups or photocopies of the originals in a different secure location. More than one person should be familiar with the procedure and storage locations; Consider how the data will be used and then enter the data into an appropriately designed database. A spreadsheet such as Microsoft-Excel is adequate for many straightforward datasets. Microsoft-Access may be a better option if the data are a subset of a bigger relational database. Copy the data on a weekly basis at minimum to a portable storage medium and keep the files in a separate location;

- Review the data and the data management system early in the process and then periodically on a regular basis. This will allow early detection of errors and inconsistencies, which can be identified and corrected before valuable information is lost;
- One competent, detail-oriented person should oversee the entire process from data collection to data entry to data storage. For some examples of datasheets that can be used during inventories and monitoring, I refer to Graeter (2008).
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Ganga basin is the largest river basin in India in terms of catchment area and drains an area of approximately 1087300 km^2 in India and Nepal, constituting 20% of the country's land mass and supporting about 43% of its population. The basin lies between 210 6'-31021' N and 730 2'- 890 6'E. The basin covers 11 states viz. Uttarakhand, U.P., M.P., Rajasthan, Haryana, H.P., Chhattisgarh, Jharkhand, Bihar, W.B. and Delhi. Ganga inhabits one of the most diversified and rich fish faunal resources of the country. Sarkar et al. (2011) reported 143 fish species belonging to 11 orders, 72 genera and 32 families. Fish biodiversity of river Ganga is currently experiencing an alarming decline due to several factors such as anthropogenic environmental degradation due to urbanization, construction of dams, abstraction of water for irrigation and power generation, pollution and introduction of exotics. Current approaches to conservation and protection of fish biodiversity of river Ganga are substantially lacking in effectiveness, and thus more effective management techniques and feasible tools are required for developing management plans for the Ganga river system aimed at a long term strategy to improve the environmental flow, restoration of fish biodiversity along with addressing the water scarcity issues.The present document addresses the assessment of fish biodiversity of the Ganga river along with conservation measures for developing suitable management and restoration plan.

1. Fish Biodiversity Assessment:

Sampling sites will be selected from entire course of river and will be covered through quarterly sampling. Estimation of fish diversity and catch data (total and individual species) will be based on samples collected from experimental fishing from the river. Habitat quality parameters will be analyzed from these sites during the same period.

Table 1.1: Format of sampling centers under NMCG project

Stretches	Site	Permanent Sampling Stations	Distance between two stations (Km.)	GPS Location
Upper stretch	1			
	2			
	3			
	4			
Middle stretch	5			
	6			
	7			
	8			
Lower stretch	9			
	10			

	11			
	12			

1.1 Assessment of fish and shellfish diversity and composition

Assessment of fish diversity and composition willbe carried out through quarterly sampling at selected sampling stations in river Ganga. Fishing activities along the sampling sites employ a wide variety of gears with several local variations. For fish diversity studies, samples from the catches of all the fishing gears (both selective and non-selective) found operational during sampling survey (seine nets, gill nets, bag nets, lift nets, cast nets, hook and line, traps, set barriers, etc) will be collected (directly from the fishers' catch, unsorted) and analyzed separately. Catch of non-selective gears are given more importance as it catches almost all the fishes present in the water column. Experimental fishing with non-selective gears will be also performed in case of less fishing activities in the area. The fishes caught will be categorized species wise, count, weight and majority of the fish species will be identified on the field itself. Length and weight of all the fishes were recorded. Unidentified fish samples were preserved in 10% formalin and taken to the laboratory for further analysis.

The fishes will be identified up to species level with the help of standard taxonomic literature (Hamilton, 1822; Day, 1889; Talwar and Jhingran, 1991; Jayaram, 1999). The fish species will be listed according to the classification scheme by Nelson (2006). The scientific name of each fish species will ascertained as per updated and revised scheme provided in the Eschemeyer Catalog of Fishes. For comparison of fish community structure between different sampling stations, c-dominance plot (PRIMER v6 PERMANOVA software package) where cumulative relative abundance/dominance of fish species (Y-axis) from a sampling zone is plotted against the increasing species rank on X-axis. The c-dominance curves for all stretches will be compared to determine whether the fish community structure exhibit any signs of ecological stress. The abundance data of the fish samples will be also subjected to cluster analysis (PRIMER v6 package of the Plymouth Marine Laboratory, U. K) to study the similarities in fish assemblage pattern among various sites. Comparisons of the present data on fish catch structure with historic data (previously reported fish catch data) at those sampling locations will be performed to understand time scale changes.Data regarding threats faced by the fish fauna were obtained from both primary (direct observations and interactions with local stakeholders and fishermen) and secondary (web based) sources. A data matrix was constructed with habitat values and fish occurrence for each of the sample stations. The

relative abundance (RA %) of fish across different sites was calculated by the following formula:

Number of samples of particular species × 100/total number of samples

1.1.1 Frequency of occurrence

Frequency distributions of the species across the rivers and sites will be plotted for studying the extent of skewness of the data sets. Species richness, as well as compositions, will be compared (across rivers) to study the extent of species shared between them and in identifying those found exclusively in particular regions in a river. The frequency of occurrence for each species (V %) will be calculated according to the equation:

$$V = ai / A \times 100\%$$

Where, ai = the number of collected samples when, some particular species was caught, A = The total number of all samples collected during the study period.

1.1.2 Catch per unit effort (CPUE)

Catch per unit of fishing effort (CPUE) is the total catch divided by the total amount of effort used to harvest the catch. The CPUE of the gill net will be calculated for each sampling sites.

1.2 Analysis of biological indices

There are many ways to assess the conservation value of assemblages (Darwall and Vie, 2005). As a consequence, there are an increasing number of indices in the literature that use various criteria to characterize the assemblages. A diversity index is a mathematical measure of species diversity in a community and provides important information about rarity and commonness of species in a community. The ability to quantify diversity in this way is an important tool for biologists trying to understand community structure. The first and obvious way to quantify biological diversity simply consists in counting the number of species (N) present at a given location during a given time period (Ricotta and Avena, 2003). Fish species diversity will be subjected to diversity analysis using different indices like species richness (S = number of species), Shannon–Wiener diversityindex (Shannon and Wiener, 1963), Simpson Index (Simpson, 1949), Species Dominance Index (Berger and Parker, 1970), Pielous Evenness (Pielou, 1966) and Jaccard's similarity index. Since most ecosystems are today threatened by the introduction of exotic species, we have calculated an index that takes into account the origin (i.e. native vs exotic) of the species.

1.2.1 Shannon-Weiner index (Shannon and Wiener, 1963)

The Shannon index, sometimes referred to as the Shannon-Wiener Index or the Shannon-Weaver Index, is one of several diversity indices used to measure diversity in categorical data. It is simply the information entropy of the distribution, treating species as symbols and their relative population sizes as the probability. The advantage of this index is that it takes into account the number of species and the evenness of the species. The index is increased either by having additional unique species, or by having greater species evenness. The value of Shannon diversity is usually found to fall between 1.5 and 3.5 and only rarely it surpasses 4.5. The Shannon diversity index occurs in situation where all species are equally abundant. Shannon diversity is the very widely used index for comparing diversity between various habitats (Clarke and Warwick, 2001). The Shannon's diversity index (H) was determined as:

$$H = -\Sigma (ni /N) \log2 (ni/N)$$

Where:
H = Shannon–Wiener index of diversity,
ni = total numbers of individuals of species,
N = total number of individual of all species.

1.2.2 Community Dominance Index (CDI)

CDI = (Y1 + Y2 / Y) x 100
Where:
Y1 + Y2 = Abundance of two dominant species
Y = Total species abundance

1.2.3 Species Richness (Margalef Index) (d)

d = S-1/ ln N
Where:
S = Total No. of species
N = Total No. of individuals of all species

1.2.4 Evenness (Pielou Index) (E)

E = H/ ln S
Where:
H = Diversity index
S = Total number of species

1.2.5 Jaccard similarity index

The Jaccard index, also known as the Jaccard similarity coefficient (originally coined coefficient de communauté by Paul Jaccard), is a statistic used for comparing the similarity

and diversity of sample sets. The species confined to any of the six selected sites will be identified as being unique, i.e. found only in concerned site. Similarity index (Sj) will be calculated as per standard methods Jaccard (1912):

$$Sj = j/(x + y - j)$$

Where, Sj is the similarity between any two communities X and Y, j is the number of common species to both communities X and Y, x the total number of species in community X and y the total number of species in community Y.

1.2.6 Trophic structure and score

Based on the feeding habitat, fishes will be classified into various trophic groups (Karr, 1991). The gut contents of fishes were analyzed and four types of trophic level of fishes were considered (planktivorous = PL, benthic feeder = BE, omnivorous = OM, carnivorous = CA) and recorded. The trophic level score (Das, 2007) indicated the relative frequency of the fish using a particular trophic level among all the trophic levels available in that aquatic system.

1.2.7 Habitat orientation and score

Based on the previous knowledge of feeding habits provided by FISHBASE (www.fishbase.org) fishes will be classified into three general groups with respect to habitat orientation: pelagic (P), generalist (G) and benthic (B). Habitat orientation score denotes the relative frequency of the fish using a particular habitat among all the habitats available in that aquatic system. A t-test will be performed for common fishes between the rivers to compare the results of the scores.

1.2.8 Similarity and dissimilarity indices to identify indicator species

Sorensen's coefficient (SC) (Sorensen, 1948) developed an index called the similarity index, which measures similarity between two habitats (habitats A and B).

$$SC = \frac{2a}{2a+b+c}$$

Where, a = number of species common for two habitats, b = number of species present in habitat B but absent in habitat A, c = number of species present at site A, but absent in site B. The index value varies between 0 and 1. Zero indicates no similarity and 1 indicates maximum similarity. Calculated Sorensen's coefficients (SC) for the fish resources were calculated between the two rivers to identify the apparent pollution indicator species.

An additional composition attribute, Bray-Curtis dissimilarity (BCD), a coefficient shown to be a robust and ecologically interpretable index of changes in species composition. BCD will be calculated using the taxa abundance data (standardized using log10 (X + 1) transformation.

The Bray-Curtis measure (B) is a measure of dissimilarity; hence 1−B is taken as a measure of similarity: where the values are in between 0 to 1.

$$B = \frac{\sum [Xij - Xjk]}{\sum [Xij + Xjk]}$$

Where, Xij = number of individuals of ith species in sample or habitat or community j and Xik = number of individuals of ith species in sample or habitat or community k. All the calculations were performed using SPSS software (16.1).

1.2.9 Conservation categories

Various methods have been developed for the conservation assessment of fishes. The major classification system used internationally for assessing the status of threat to each species is that adopted and developed by the World Conservation Union or International Union for the Conservation of Nature and Natural Resources (IUCN). The Red Data Book categories are used to indicate the degree of threat to individual species in their wild habitats (IUCN, 2008). Distinctly threatened species are characteristically those fish belong to very defined taxonomic units of restricted geographic range, and appears to be particularly sensitive to one or more human threats and those populations or range which have undergone a significant decline and seems likely to continue. Following different categories of threat status will be addressed;

Extinct (EX)
A taxon is extinct when there is no reasonable doubt that the last individual has died.

Extinct in the Wild (EW)
A taxon is extinct in the wild when it is known only to survive in cultivation, in captivity or as a naturalized population (or populations) well outside the past range. A taxon is presumed extinct in the wild when exhaustive surveys in known and/or expected habitat, at appropriate times (diurnal, seasonal, annual), and throughout its historic range has failed to record an individual. Surveys should be over a time frame appropriate to the taxon' s life cycle and life form.

Critically endangered (CR)
A taxon is critically endangered when the best available evidence indicates that it meets all the criteria' s for endangered, and it is therefore considered to be facing an extremely high risk of extinction in the wild.

Endangered (EN)

A taxon is endangered when the best available evidence indicates that it meets any of the criteria for endangered, and therefore considered to be facing a very high risk of extinction in the wild.

Vulnerable (VU)
A taxon is vulnerable when the best available evidences indicate that it meets any of the criteria for vulnerable, and it is therefore considered to be facing a high risk of extinction in the wild.

Near threatened (NT)
A taxon is near threatened when it has been evaluated against the criteria but does not qualify for critically endangered, endangered, or vulnerable now, but is close to qualifying for or is likely to qualify for a threatened category in the near future.

Least concern (LC)
Taxon is least concern when it has evaluated against the criteria and does not qualify for critically endangered, endangered, and vulnerable or near threatened. Widespread and abundant taxa are included in this category.

Data deficient (DD)
A taxon is Data Deficient when there is inadequate information to make a direct, or indirect, assessment of its risk of extinction based on its distribution and/or population status. A taxon in this category may be well studied, and its biology well known, but appropriate data on abundance and/or distribution are lacking.

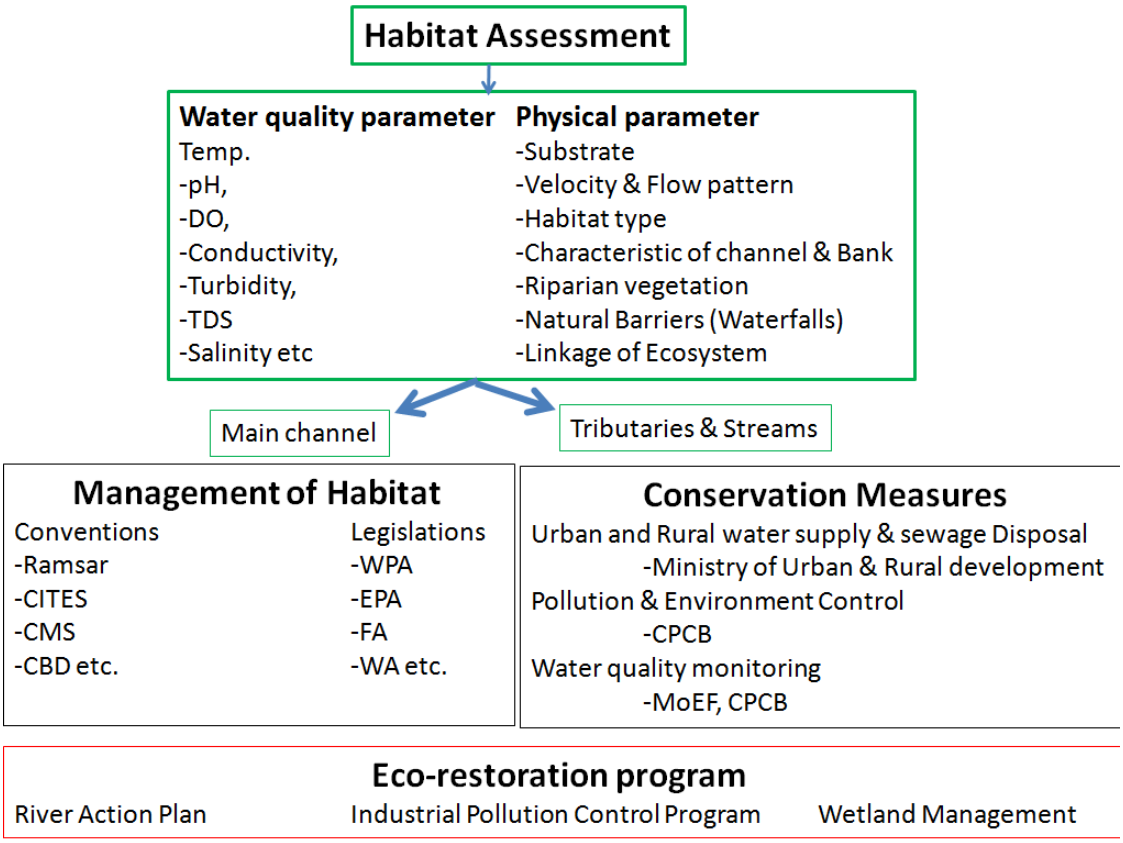
Not evaluated (NE)
A taxon is Not Evaluated when it is has not yet been evaluated against the criteria. In the present study, the conservation categorizations of the fishes will be assessed as per Lakra and Sarkar (2006).

1.3 ASSESSMENT OF HABITAT PREFERENCE PARAMETERS (ENVIRONMENTAL VARIABLES)

Collection of physiochemical parameters

Environmental variables were measured each time in the same site where fishes were sampled. Fifteen environmental variables were used to interpret the variation in species composition (Table 1). The following physical and chemical variables were measured: temperature (^{0}C), Turbidity (NTU), stream velocity (cms-1), conductivity (l mhos cm-1), total dissolve solid (TDS) (ppm), dissolve oxygen (DO) (mg l-1) and pH. Water temperature, conductivity, pH, TDS, DO were measured by Cyber Scan Waterproof PC 300 multiparameter at the sampling locations. Water velocity was measured by flow meter (JDC electronics SA; Switzerland). These variables were measured at 7, 13 and 17 h in sites where gill nets were used without disturbing the fish. In sites where we used electrofishing, these

environmental variables were measured before sampling the fish (7 and 13 h). The total percentage of each substrate class was calculated using transects perpendicular to the flow within each sampling sites. Six to eleven evenly spaced transects for each sampling length were used to survey physical habitat at each site. The dominant substrate material for each sampling site was determined by inspection and striking the river bottom with a bamboo pole. Substrate classes included sand (0.06–2 mm), gravel (2–64 mm) and cobble (64–250 mm) according to Bain and Stevenson (1999). Overhanging vegetation and riparian human influence (i.e., rowcrop agriculture, rangeland and rip–rap) was visually estimated at each transect on each bank. Observations were categorized into ranks (0–1) i.e., 0 for absent and 1 for presence. The mean of ranks was averaged across all transects for each site.For habitat preference of fishes, physico-chemical (water and soil quality) as well as biological parameters (Plankton, periphyton and benthos availability) has to be understood clearly. Collect subsurface water and soil sample from bottom of three different sites (Right bank, Left bank and Middle of the river) of each selected station for analysis of every individual parameter in two replicates. For determination of different soil parameters, bottom sediment was collected using Peterson Grab, mixed thoroughly and dried at room temperature in shade. The dried sediment was ground, strained through 40, 60, 80 and 100 No sieve separately and kept in plastic packets for analysis of different soil parameters. Soil organic C was analysed with the soil sample filtered through 100 No. sieve. Heavy metal was estimated with the sample filtered through 80 No. sieve. All the other soil parameters except texture were estimated using the sample filtered through 60 no. sieve. Soil texture was estimated using the soil sample sieved through 40 no. sieve. Parameters are analysed following standard methods (APHA., 2005).



Flow chart of Habitat assessment, management, conservation and restoration

FISH DIVERSITY AND ABUNDANCE DATA SHEET

Taxonomical classification			Station-wise abundance (Total Nos)								
Order	Family	Fish species	Station1	Station2	Station3	Station4	Station5	Station6	Station7	Station8	Station9

INDEX OF BIOTIC INTEGRITY

Family	Species	Conservation status	Dwelling habit	Trophic level	Tolerance level	Commercial value	Relative abundance (%)				
							Station1	Station2	Station3	Station4	Station5

DATA REPORT SCHEDULE FOR WATER CHEMISTRY (one)

Stations	Months	Velocity (m/s)	Depth (m)	Wetted perimeter (%)	Substratum	Air temp (°C)	Water temp (°C)	Transparency (cm)	Turbidity (NTU)	Sp. Cond. (mS/cm)	pH	DO (ppm)	TDS (ppm)	Total Alkalinity (ppm)	Total Hardness (ppm)	Chloride (ppm)	Salinity (ppt)

DATA REPORT SCHEDULE FOR WATER CHEMISTRY (two)

Stations	Phosphate-Avail P (ppm)	Phosphate-Total P (ppm)	Nitrate-Avail N (ppm)	Nitrate-Total N (ppm)	Silicate (ppm)	B.O.D (ppm)	COD (ppm)	Chlorophyll a (mg/m³)	Chlorophyll b (mg/m³)	Chlorophyll c (mg/m³)	Total Chlorophyll (mg/m³)	GPP	NPP	CR

1.3 Statistical analysis

A data matrix was constructed with habitat values and species presence and absence for each of the 50 sample sites. Each habitat variable was represented by a mean when repeated habitat measurements were made in different seasons. Principal component analyses (PCA) were performed on the 15 environmental variables to reduce the dimensionality of the data and to examine initially the influence of habitat characteristics. All variables were log10(x+1) transformed before analysis to better meet the assumptions of normality. Variable loadings [0.25were considered important in structuring the stream (Chatfield and Collins, 1980). Frequency of occurrence was calculated for each species separately as total number of sampling stretches (50 sites) containing the species. Percent of times observed in the sampling sites out of the five sampling was calculated for each species.

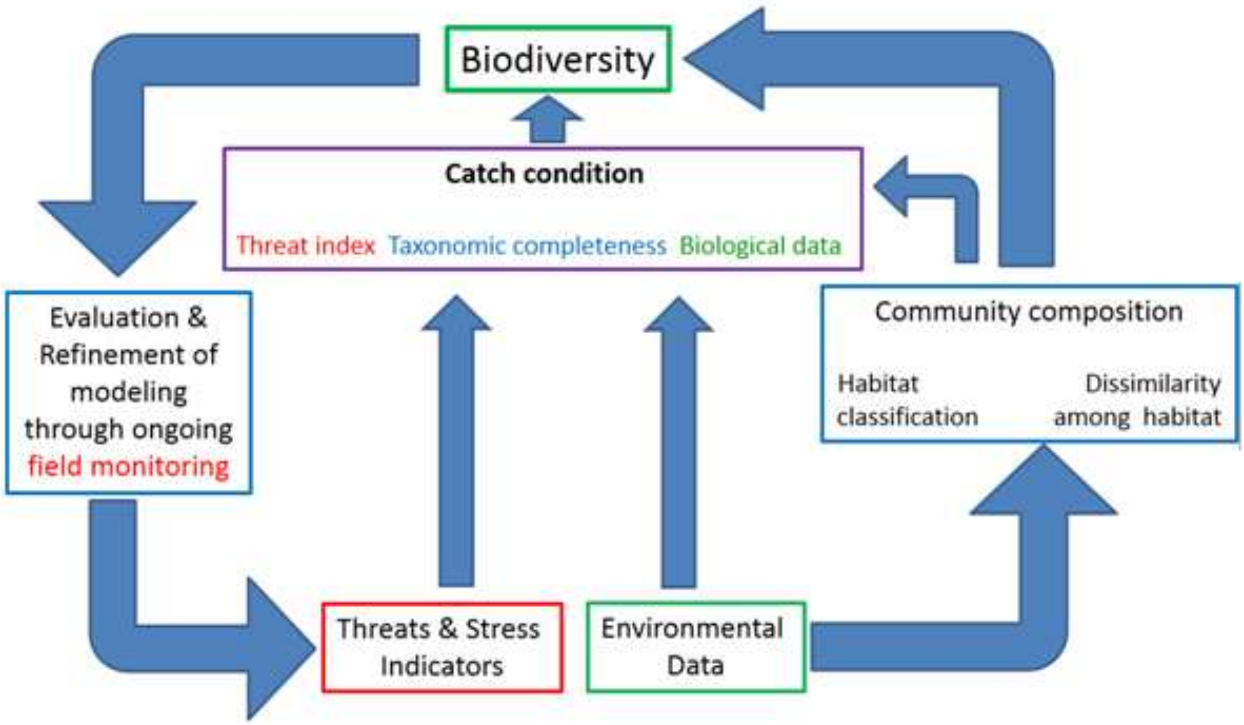
Canonical correspondence analysis (CCA) (terBraak, 1986) was used to identify the relationships of environmental variables with fish assemblage. A stepwise forward selection and Monte Carlo permutation test (1,000 random permutations) were used to determine environmental variables that significantly (P\0.05) explained variation in fish assemblage data sets. Partial CCAs were used to determine the variance explained by individual variables after the removal of variables with inflation factors [10 (terBraak and Smilauer 2002). All statistical analyses were conducted using the Multivariate Statistical Package (MVSP) trial version 3.1 (Kovach, 1999), SPSS version 16.0 and CALIBRATE 1.0.

Conservation priorities for measuring change in fish biodiversity:

The current conservation priorities for freshwater fishes and fish habitat are:

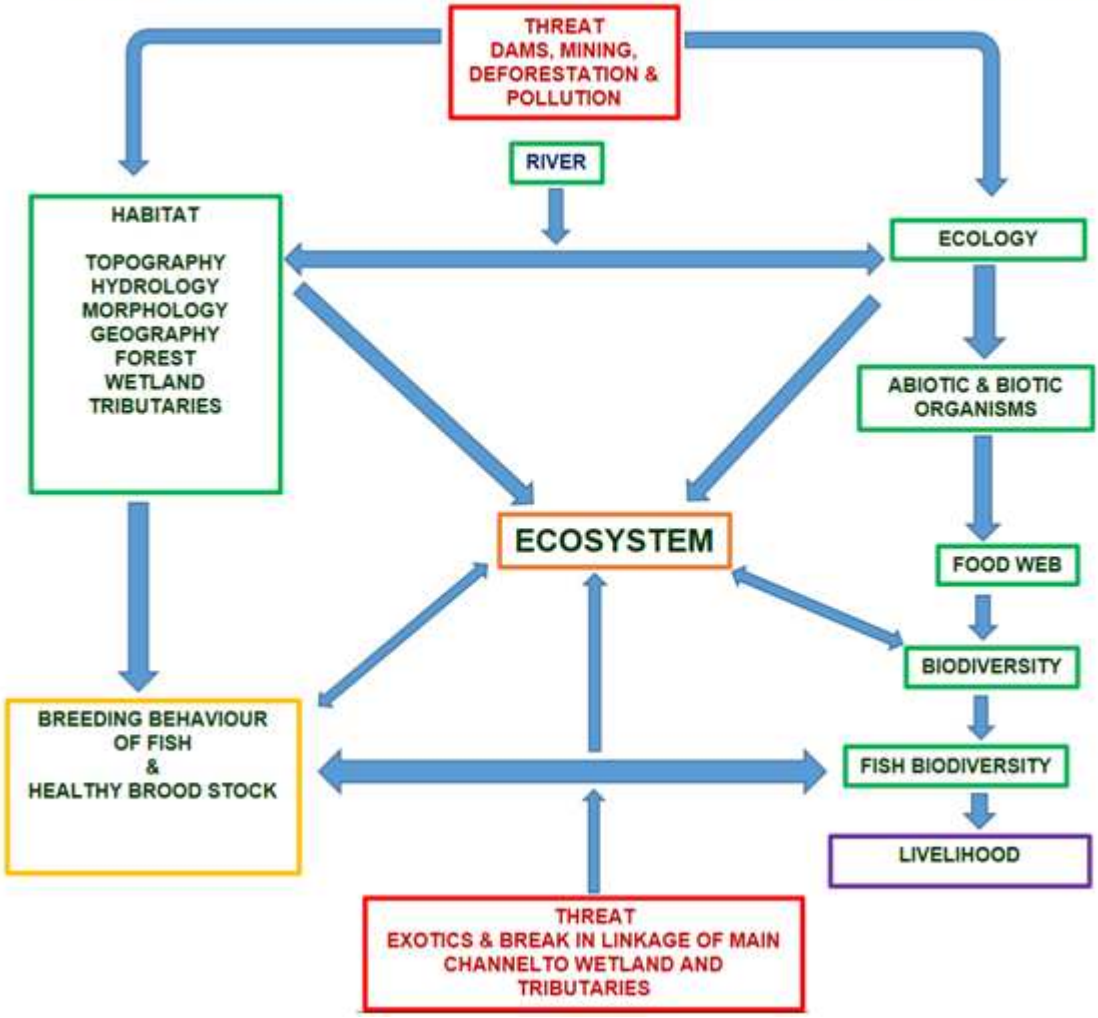
i) Cataloguing fish biodiversity, along with indices of abundance;

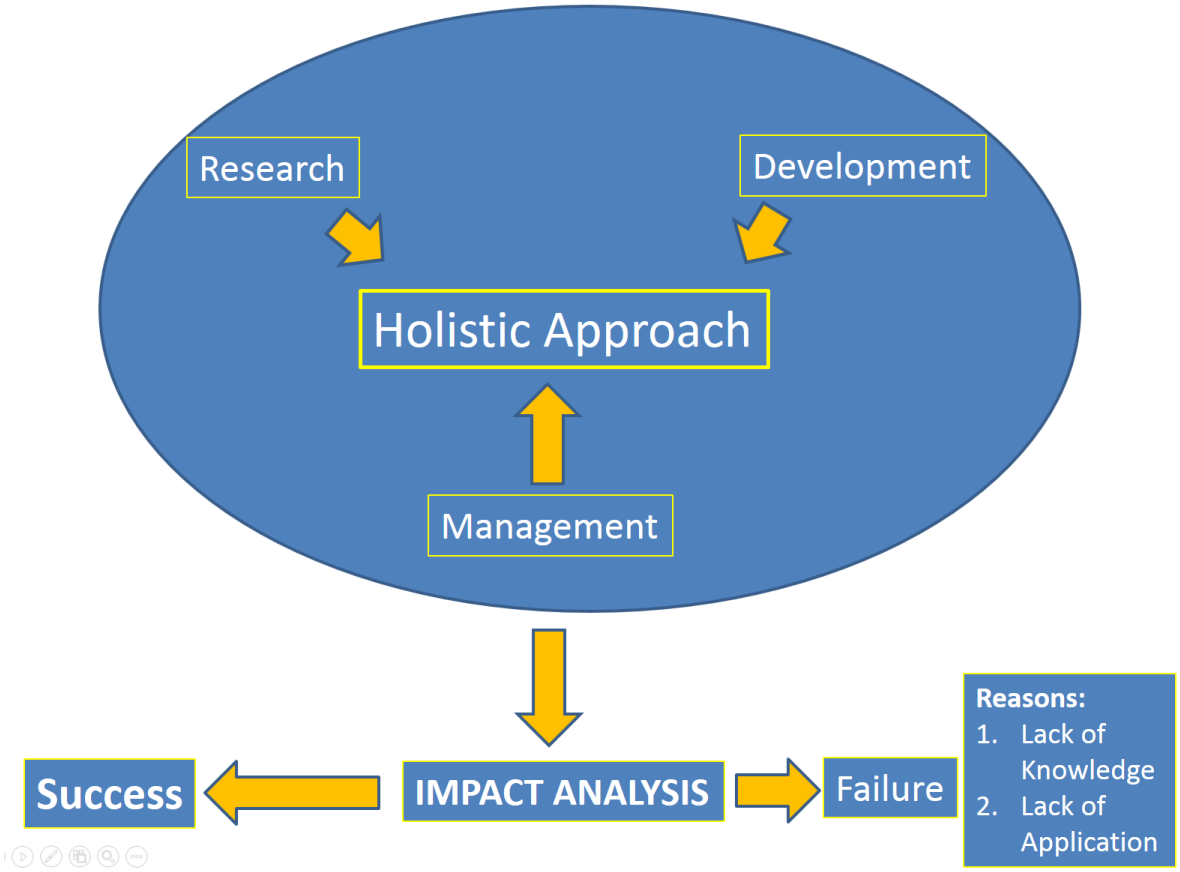
ii) Repeat Red List assessments at 5–10 year intervals;
iii) Complete DNA barcoding library of all fish species;
iv) Develop standard molecular markers to widely assess intra-population diversity;
v) Develop models to predict susceptibility of fish species to climate change impacts;
vi) Establishing reports with updated threat data every 3–5 years and additional sampling to improve spatial resolution in data-poor regions.
vii) In situ conservation of endangered species through species specific habitat requirement studies by declaring Sanctuary or Protected Area (Non Fishing Zone) after certain range;
viii) Captive breeding and Ranching as a source of Restoration & Conservation;
ix) Genetic management of natural and farmed stocks due to genomic introgression;
x) Sustainable harvesting of natural stocks especially in hill stream or isolated standing waters, where destructive fishing through dynamiting or poisoning is a major threat;
xi) Incorporation of new candidate species into the aquaculture and ornamental fish industries to for diversify aquaculture production;
xii) Formulation of guidelines on the introduction of exotic species and their quarantine;
xiii) Regulation of environmental flow (Joshi et al., 2014);
xiv) Ecosystem connectivity
xv) Issues of Fishers (Socio-Economics); and
xvi) Awareness campaign is central responsibility under NMCG, involving fisher communities at a larger scale with an aim for: Controlling on destructive fishing methods, mesh size regulation and ban period implementation.



(Source: Turak et al., 2016)

Conservation & Restoration Model





Impact Assessment

Introduction

Small size of the invertebrates enables them to exploit very small and specific features in the environment, known as microhabitats. Many species, especially insects, inhabit different microhabitats during various stages of their life cycle. This implies that sampling techniques used for them needs to be devised on a finer scale than those used for many vertebrates. Data may need to be collected from different microhabitats in a region or from the same habitat over a chronological time period. A vast array of sampling methods has been devised for sampling invertebrates, which may be large or expensive pieces of equipment and consist of modifications of existing trap designs. The activities of most invertebrates, especially insects, often tends to be strongly influenced by weather conditions and time of day. The level of activity dictates the habitat of the individual, how easy it is to locate, catch and trap the individual. This implies that the weather conditions and time of day need to be standardised through the duration of experiment. Traps operate on the active entry of the individuals, which reflects abundance, and susceptibility of being caught. The traps that use attractants indicates the distance to which the attractant works for the particular species, which might vary with difference in weather conditions and locations. Catching invertebrates often includes difficulty in identification, and death of trapped individuals, which may further affect the population, especially if they are sexually mature reproductive individuals, as in the case of dragonflies, butterflies and crickets.

Temporarily storing live invertebrates

Live invertebrates should ideally be stored in glass or plastic specimen or jam jars, or clear plastic bags, along with a small amount of vegetation for terrestrial invertebrates so that they have something to attach them to during transport. Imitation of the natural habitats of the individuals should be made as far as possible, in terms of climatic conditions as well as the environment of their habitats which includes factors like temperature, moisture content, salt ratio etc. Containers should be kept in a cool, dark place to reduce stress on their occupants. Trapped individuals should also be released in the same place they were caught, and in the case of groups like bees or other such nectar- or pollen-feeding insects, near flowers so that they can feed themselves if exhausted.

Killing and preserving insects

Killing and subsequent preservation can be done by dropping them into 70% alcohol solution. It is often better to ‘fix’ the individuals beforehand, particularly if they are to be used in reference collections. Fixing is the process of stabilising the protein constituents of the body tissue to help

preserve them in the condition they were when the animal was still alive. This can be done using a wide range of chemicals.Insects can also be killed by exposing them to ethyl acetate fumes, which is best done in a 'killing bottle', which is essentially a glass bottle containing a layer of set plaster of Paris onto which a few drops of ethyl acetate are dripped. The PoP can also be replaced by a piece of crumpled tissue paper at the bottom of the bottle. When using alcohol solution to preserve the bodies, the glass containers should be properly sealed because alcohol can quickly evaporate. For storage for more than 5 years, it is advisable to add 5% glycerol to the solution to prevent the specimen from becoming brittle or completely drying out, should the alcohol evaporate. Butterflies and moths should be preserved by pinning them to avoid damaging the scales of their wings. Best way to label specimens preserved in alcohol is to write with pencil on a card and place it in the bottle alongside the specimen. Invertebrates should only be killed when there is no other way of sampling.

Direct Searching

All invertebrate groups

Method

Easiest way to find invertebrates is often to just look for them in suitable microhabitats. Many terrestrial invertebrates require sheltered, moist microclimates, and can be found under stones, logs, bark, around the base of plants, in crevices in walls or rocks, in leaf litter, nests, dead and decaying matter and dung. When searching such areas, it is important to return stones or logs to their original position, to prevent the animals underneath from becoming desiccated. Care should always be taken to cause minimal disturbance to the habitat. Often, many invertebrates can be found by cutting off tussocks of grass at the root level and then shaking and cutting it open under a light and on a white surface. Slugs and snails are more conspicuous during wet weather, especially at night, while scorpions are best searched for using a torch with an ultraviolet-emitting bulb. More active insects, like dragonflies, damselflies, butterflies and moths, often require a more active search and capture. A pooter or fine moistened paintbrush is useful for quickly picking up small insects and arachnids. Direct searching is also the simplest method for finding less active aquatic species. The most productive places to search include on or under stones, amongst aquatic vegetation, and among sticks and roots of marginal vegetation. The most effective way of finding aquatic invertebrates is to place the aquatic weeds in a water filled, covered bucket overnight. The subsequent oxygen depletion and slight fouling of the water encourages most of the hidden invertebrates to come to the surface.

Seashore invertebrates can be found by searching the sand bars between high tides, especially during spring tides, which allow the maximum range of foreshore to be searched. The most profitable areas to search are among the seaweed fronds, stems and holdfasts and under stones in rock or tidal pools.

Many aquatic invertebrates are delicate and should be handled very carefully. Mark-recapture method is often used for some invertebrate taxa with hard exoskeletons, where the marking is done on the exoskeleton using oil-based enamel paint, or marking wings of moths and butterflies with felt tip pens or gluing numbered tags onto the carapace of crabs.

COUNTING NUMBERS PER UNIT EFFORT

Timed searches are often used to search invertebrate fauna of ponds. This method can also be used in terrestrial habitats, mainly for relative estimates of conspicuous taxa like butterflies at different heights in a rainforest canopy.

COUNTING INDIVIDUALS PER UNIT OF VEGETATION

Invertebrates and plant galls can be searched for on individual leaves, stems or entire plants. It can also be done by extracting standardised samples of foliage from trees or bushes and placing them in polythene bags or plastic bin liners, with care not to dislodge the invertebrate fauna attached. The standardisation can be done by (a) counting number of leaves or buds per sample, after checking for invertebrates or (b) by weight. The sample must then be thoroughly checked for specimens under light and a suitable background. Densities of invertebrates and/or galls within plant parts can be obtained by removing the required parts and dissecting them.

COUNTING NUMBER OF INDIVIDUALS PER UNIT AREA

Conspicuous invertebrates can be surveyed by thoroughly searching in a defined area. Smaller areas can also be defined within habitats by, for example, placing random quadrats throughout it. These can be searched for immobile taxa or casts or holes made by polychaetes or bivalves. Worm casts tend to collapse at varying rates depending on moisture content in the substrate, and thus, should be counted within a standard time. In case of mobile taxa, it is better to leave the quadrats undisturbed for some time before searching it another method is to use a 'box quadrat' to help confine individuals while they are being counted. Counting exuviae is a useful method for indexing the productivity of insects that produce conspicuous exuviae in areas that can be thoroughly searched.

COUNTING ALONG TRANSECTS

Mainly developed to obtain quick estimates of butterfly colonies for use in surveys. It can also be used to monitor other large, conspicuous invertebrates. In aquatic habitats, transects have been mainly used to obtain estimates of sessile invertebrates on the foreshore. When recording estimates with transects, it is essential to carry out the transects at similar times of the day and under similar weather conditions.

Advantages and disadvantages

It is often the most useful for surveying invertebrates since it allows suitable habitats and microhabitats to be quickly and easily investigated. Direct searching has the advantage of being selective. It allows the capture and release of individuals unharmed after being identified. It will however often be less efficient in terms of number of individuals caught per time spent in the field than trapping methods.

Counting on field is fast and doesn't require any equipment but it requires prior identification knowledge. Removal of vegetation is obviously destructive, and may require the transport of large amounts of vegetation. It can also be time-consuming. Counting conspicuous invertebrates or larval aggregations is quick and easy but only where the taxa occur in a very limited range of density of being between minimum density needed to obtain meaningful results and not high enough that counting becomes impractical. Searching for exuviae may be relatively quick, but the whole process of waiting throughout the emergence period can prove to be very time-consuming.

Biases

Direct searching is most likely to locate more visually obvious, active and large species, although difficulty in catching large, active flying insects may result in under-recording of these species. Small and cryptically coloured species are likely to under-recorded. Disturbance during searching might cause some individuals to escape, or being counted multiple times. Exuviae might be lost, leading to underestimation of the productivity of the site. Variable rates of collapse of worm casts may bias counts

Water traps

Flying insects, mainly flies and Hymenoptera

Method

Many flying insects are attracted to certain colours and can be attracted to and caught in coloured water filled bowls. Yellow bowls are the best for attracting both flies and Hymenoptera, whereas white bowls attract flies but act as strong repellents for Hymenopterans. 'neutral' coloured bowls, like brown, grey or blue have the least attractant (or repellent) effect on insects, and so reduce the selectivity of the sampling.The species composition attracted by the bowls also differs with the height of the traps. Total trap catches are highest when traps are set just above the level of the surrounding vegetation. In woods, a wide-nesh gauze needs to be used to cover the traps to prevent leaves falling into them and affecting the attraction of the trap.Traps should be cleared at least once a week, and the trapped invertebrates can be removed by passing the contents through a muslin cloth, failing which the contents will start decaying unless a preservative is used, which in turn might affect the attractiveness of the trap.

Advantages and disadvantages

They can be used in virtually any habitat. But they are virtually impossible to protect from grazing stock, which might use them as drinking troughs. Fencing off the traps, however, might affect the vegetation in the immediate vicinity of the fence, and thus affect the catch. Insects caught in the traps are sometimes also preyed on by birds.

Biases

Number of insects trapped will depend on their activity and their attraction to the traps, as well as their abundance.

Flight interception traps

Flying insects

Method

These traps work by blocking the flight path of the insects with a fine black netting. Blocked insects then drop down into a collecting tray laid down beneath the trap, or are guided upwards into a collecting bottle.

These traps are best sited in areas frequented by large numbers of flying insects like along woodland edges or ridges or near hedges. These traps can be checked as infrequently as once a week.

Advantages and disadvantages

They are large and conspicuous, thus, prone to disturbances by passers-by. They are rarely used to compare numbers of insects between sites or at the same site over time, because their size tends to make replication impractical.

Biases

The interception traps only catch few small active insects, and proportionally more heavy, cumbersome flying insects, such as large beetles. Malaise traps are better at catching smaller, more agile flying insects.

Light traps

Mainly moths but also other nocturnal flying insects

Method

Mainly nocturnal flying insects, particularly moths, are attracted towards light, particularly to the ultraviolet end of the spectrum. They can be actively caught as well as coaxed to enter a trap.The simplest trap consists of a light hanging on a cable outside a building. Any bright white or bluish light is suitable, but a high-pressure mercury vapour bulb is the best, which run on direct current which

means that an adapter is necessary to run them. In addition to being very bright, these bulbs emit ultraviolet light. The effectiveness of the trap can be increased if the light has a white background. Catches can also be increased by using a moth trap, which can be left running for long periods without supervision. Light traps attract most moths on warm, overcast nights with a little wind, especially with thunder.

Advantages and disadvantages

These are capable of catching a large number of moths under favourable conditions. However, these numbers are subject to fluctuation depending on the weather conditions and so can't be used to monitor moth numbers without long term usage.

Biases

Catches reflect the activity of individual species. The insects caught will most likely enter and remain in the trap.

BIOLOGICAL SAMPLE COLLECTION

Ajit Kumar, Prabhaker Yadav and Sandeep Kumar Gupta

Introduction: In conservation genetic, sample collection is the crucial step for identifying the species, home size range, and population dynamics as well as to generate a DNA reference database. The sample collection procedure will vary according to specimen type and intended analyses, but all procedure should be carefully designed and documented. Prior to initiating a study that will involve the collection of biological samples, many decisions need to be taken that will affect the quality of the samples and outcomes of the study. If the samples are not properly stored and treated, DNA can be degraded. The degradation rate can vary from sample types to species depending upon the samples collection procedures. Here, we provide best methods for sample collection and storage for different types of biological samples of aquatic fauna.

1. Samples collections

The samples collections will vary depending on the test to be run. What tissues and fluids are collected will vary depending on the size and age of species, the purpose for which the test is being performed, and the requirements of the test used by receiving laboratory.

Samples collection procedure is mainly of two types: Invasive and non-invasive method.

In non-invasive methods, the samples were collected without harming the animals. This method does not require much expertise. However, non-invasive collection method suffers from low DNA yields, quality, and usability for the different segments of populations. Examples: Feces, shed hairs, eDNA, dead remain tissue and bones.

In invasive methods, the samples were collected directly from the live animal body parts. This method is painful and required expertise and has handing infrastructure expensive. This collection technique provides high and contaminated free DNA. Examples: Blood, tissue, biopsy, plucked hairs.

2. Types of Samples

2.1 Blood

The blood is ideal sample to extract the best quality of DNA. Yet it is expensive to collect and involves lots of efforts and expertise to be obtained. The best way to preserve blood is either using vacutainers with ethylene-diamine-tetraacetate (EDTA). If using vacutainers, make sure to mix well so that the EDTA gets in solution, and keep refrigerated. We usually only need very little blood to be able to extract DNA, therefore about 100µl of blood should be more than sufficient for genetic purposes. In fishes blood can be collected by caudal venipuncture or by using vascular sinus located caudal and slightly ventral to the dorsal fins (Image 1a). In turtles where blood sampling is difficult due to hard shell, therefore blood is

usually drawn from the dorsal cervical sinus in the neck or from the femoral venous plexus in the hind flipper (Image 1b).

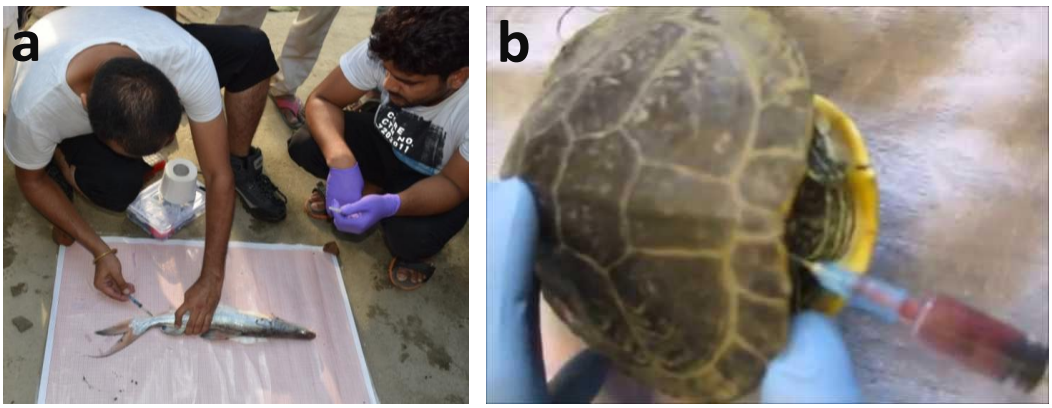


Image 1: Blood sample collection from (a) fish caudal vein (b) turtle.

2.2 Tissue Samples

Fresh tissue consistently provides the highest yield and quality of DNA. Tissue collection is always depending on the size of organism to be studied. The skin is sampled using a surgical blade or biopsy punch to collect tissue from the top few layers of the epidermis (Image 2a). A small portion of about 100-200 mg sample provides excellent DNA in both quality and quantity. The best ways to preserve tissue samples is by immersing it in 70% ethanol and wrap the collection vial cap with parafilm to avoid leakage, and label the tubes appropriately. Tissue samples preserved in this manner can be kept at room temperature (Image 2b).

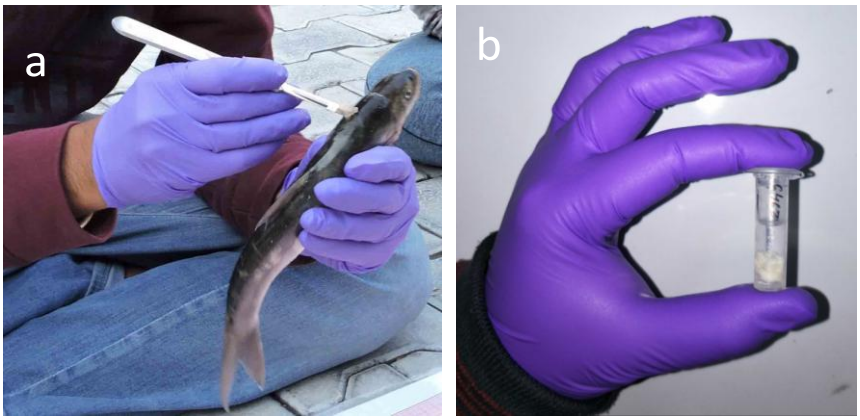


Image 2: Tissue sample collection from (a) fish (b) tissue stored in 70% ethanol

2.3 Buccal and Cloacal Swabs

Buccal and cloacal swabs have been successfully used to obtain genetic material from various aquatic species such as fish, turtles, frog, juvenile gharial and crocodiles etc. To open the turtle's mouth, stainless steel mouth pry bar will be used to hold the mouth open. The epidermal cells will be collected from the mouth or from the cloaca by gentle scraping with swabbing bud of approximately 5 mm for about 10 sec (Image 3). Immediately after

swabbing, the swab will have to be placed inside 15 ml conical tube and stored in ice until transfer to a laboratory. Ethanol preservation will not be used in swabbing techniques because the liquid absorbs by the buds which dilute the cells and affect the DNA yield.

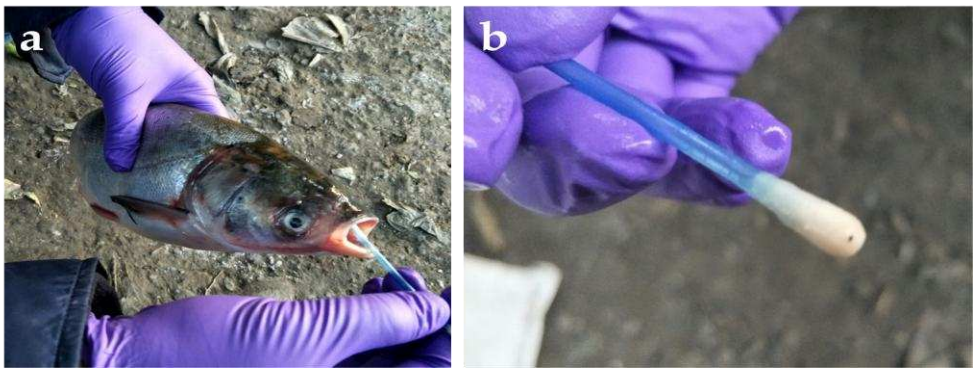


Image 3: (a) Buccal swab sample collection in fish (b) swab bud sample

2.4 Biopsy

The biopsy is recent techniques to obtain small tissue samples from free-ranging large size animals using a remote biopsy system. This technique required much expertise and special permission. The remote biopsy system consists of caliber rifle with a barrel, a pressure valve system, and a biopsy dart needle. Biopsy darts have a hollow body and a steel biopsy tip that is beveled inwards. While manual biopsy is the collection of tissue sample with the help of punching device (hollow needle) (Image 4). Obtaining tissue from a turtle, fish, and dolphin DNA analysis via tail or fins biopsy is a safe, effective and gentle procedure. The tail biopsy procedure must be performed using clean gloves and a sterile needle or sharp scalpel, scissors, or razor blade. The tail skin should be disinfected with alcohol prior to incising the tip (Do not use iodine solutions because they may interfere with DNA analysis). During the biopsy procedure, bleeding should be controlled using local pressure and sterile gauze.

Image 4: Biopsy punching  **device**

2.5 Carapaces and scute

A carapace is a dorsal (upper) section of the exoskeleton or shell in a number of animal groups, the tissue samples can be collected along the posterior marginal scutes located on the carapace (Image 5a). A scute referred as bony external plate or scale overlaid with horn, as on the shell of a turtle, the skin of crocodilians, and the feet of birds. In hard shell turtles, the shells are mostly made up of keratin, they are built similarly to horn, beak, or nail in other species. Scutes sample can be collected with 6 to 10 mm biopsy punches from the posterior margin of the lateral scute (Image 5b).

Image 5: (a) carapace sampling for hard shell turtle (b) Scutes of crocodile

2.6 Feces

The collection of feces for genetic studies is a completely non-invasive procedure. Fresh feces contain good amount of epithelial cells which serve as a source of DNA for genetic analysis (Image 6). In aquatic species such as fish and dolphins, feces are difficult to collect because it dissolves in water however; the other species such as turtles, frog, juvenile gharial and crocodiles feces are easy to collect. The single individuals will place in beaker or rectangular box (vary with body size) for 2-3 hrs, then released it to the same place. Then swab the surface with clean sterile cotton or ear bud and immediately after swabbing, place the swab inside 10-15 ml conical tube and placed it on ice or deep freezer (-20ºC) until transfer to a laboratory. In order to prevent with cross contamination among individuals, gloves and beaker or rectangular box need to be change each time.

Image 6: Water contains feces of turtle

2.7 Environmental DNA (eDNA)

eDNA is DNA that is collected from the environment in which an organism lives, rather than directly from themselves. The collection and analysis of eDNA from water samples is an effective method of determining the presence of aquatic organisms such as dolphins, fish, amphibians, reptiles and other taxa. Generally, eDNA will exponential decay over time, and can be collected through a water sample which is then analyzed to determine if the target species of interest have been present in the waterbody. In the case of the dolphin, the samples will be immediately collected after the animal defecated. The fresh samples will be individually collected in plastic bags, and frozen until DNA extraction will possible.

3. Materials required

Weighing machine (upto 10 kg), dissection tray, scale, scissors, forceps, surgical blades and scalpel, disposable syringe with needles (1.0 ml & 5.0 ml), sample collections vials (2ml, 5 ml, 10 ml), falcon tube (25 & 50 ml), EDTA vials, Ethanol, silica beats, distilled water, heparin, cryo box/ice box, cryo tag, ceiling tap, parafilm, GPS, camera, plane sheet, data sheet, gloves, aluminum foils, tissue roll, cotton, dishtowels, swapping buds, fine permanent black marker
small bucket (optional), storage bags and desiccant.

4. Storage of blood/tissue/skin/feces samples

a. Vacutainer tubes (blood may be collected as usual for routine purposes) and stored at 4ºC until handover to the lab.

For tissue/skin and feces

b. Always use screw capped small vial

Note: Do not use more than 100 ml/gm capacity

b1. Fill approximately half of the vial with Silica gel (5-8 mess size)

OR

b2. 2/3rd volume of the container with 70% ethanol or absolute ethanol (C_2H_5OH)

Note: 70% ethanol can be prepared by mixing of 75ml ethanol with 25 ml of distilled/mineral or packed water. In ethanol sample can be stored at room temperature for many days.

c. Place a circular paper piece over the Silica Gel (do not put anything for ethanol)

d. Place small piece of meat (10-20gm)/skin piece (3×3 cms)/fresh feces (15-20gms.) Over the filter paper (or directly dip the sample in ethanol) and make airtight with the cap. Please write the species and place, date of collection of the sample on the vials.

Note: In case of ethanol preservation cape should be sealed properly with parafilm to avoid the leakage. Donot use formalin as a preservative for genetic analysis because it degrade the DNA.

4. Detail information about the collected biological samples for genetic study

S. No.	Species Name	Sex	Sample code	Sample Type *	Sample location	GPS location		Date of collection	Remark
						N	E		

*** T=Tissue; S=Fecal; E=Egg shell; F= feather; B=bone; H=hair; C=claw**

Point Count Datasheet

Group Name…….Point I.D……..... Date……GPS Lat…… (N) Long…… (E) Start Time……..... End Time…..... Weather Condition…… Remarks……

S.no	Species	Count	*Distance Zone	Time		Numbers			Habitat	Activity	Comments
				0 to 5 minutes	5 to 10 minutes	M	F	J			

***Distance Zone 1 = 0 to 50 meter, 2 = 50 to 100 meters, 3 = 100 to 150 meters, 4 = 150 to 200 meters**

Line Transect Data sampling Data Sheet

Group Name……..... Date…….....…….Start Time……End Time……..
Transect Id…… Start GPS Lat…… (N)
Long……(E) End GPS Lat…… (N) Long…… (E)
Total Length (Meters)……. Weather Condition……
Remarks……

S.No	Time	Bird Species	Total No's	Total			Transect Bearing	Sighting Angle and Bearing		Angle*	Habitat Type	GPS Location (Bird Sighting)
				M	F	J		Bird Bearing	Distance			

***If the sighting angle is between 90˚ & 179˚, recalculate it as 180˚- sighting angle; If sighting angle is between 180˚ & 270˚, recalculate it as sighting angle - 180˚; If sighting angle is between 271˚ & 360˚, calculate it as 360˚ - sighting angle. PERP DISTANCE=Sin(Sighting Angle)×Distance to animal**

Introduction

Most plant communities consist of individual plant arranged on a surface (e.g. soil or rock). These plants are sessile; i.e they 'sit still and wait to be counted'. This makes some surveying jobs simple. For instance, it is very easy to wander through the vegetation and make a species list. However, species and individuals within species often vary enormously in size causing problems in selecting the best measure of species abundance.

Methods applicability:

Table: Swing applicability of methods (* usually applicable + often applicable and ? sometimes applicable)

Methods	Trees	Shrubs	Herbs and grasses	Aquatic species	Bryophytes	Fungi and lichens	Algae
Total counts	+	+	?				
Visual estimates	*	*	*		*	*	+
Frame quadrats	+	+	*	+	*		+
Transects	*	*	*		?	?	
Point quadrats			*		*		
Plotless sampling		+	?				

Methods

Total count:

This method is used to assess the density of large or obvious plants that are at low density. This technique is so simple it might be overlooked. Every individual of a species or a number of species in the study are is counted.

Visual estimates of the cover:

It defines the cover of species in any vegetation. Visual estimates are made of the cover of the species wither in the whole study area or in sample plots, such as frame quadrats. Different measures can be used. The simplest is the classification: dominant, abundant, frequent, occasional or rare. These classes have no strict definition and you must decide on your own interpretation. Percentage cover can be estimated by eye either by creating your own percentage classes or by following some good Authors'.

Frame quadrates:

Often these simply called quadrats. They are used to define sample areas within the study area and are usually four strips wood, mental or rigid plastic which are tied, glued, welded or bolted together

to form a square. It helps in defining the cover, density, biomass and frequency of species in any vegetation. Qudrates are placed in the study area according to your sampling design and different measures can be used to survey the vegetation. Density is measured by counting the number of individuals of the study species, frequency is measured using percentage of the species.

Transect methods:

The line transect or line intercept methods involves using the actual transect line as survey implement. A simple measure is count the number of plants of species that touch the transects.

Point quadrates:

A point quadrate is thin rod with a sharpened tip and should usually be made of metal for rigidity and strength. Good materials are thick gauge wire, welding rod, knitting needles or even bicycle spokes. The data recorded from the point quadrat are only the presence and absence.

Plot less sampling:

A number of plotless sampling or point to object, methods are possible. It incluses nearest individual method and the point centered quadrate method.

Data Sheets

Plant vegetation								
	Plot N°_____ Square N°_____							
LO CATION :								
LO CALITY:								
Lat. S L		**Long. W E**				**elevation (m)**		
Vegetation Type:								
Soil Description:								
Plant Description:								
Date:								
Point No.	**Life form**	**Diam. 1 (cm)**	**Fam. Diam. 2 (cm)**	**Scientific name**	**Width (cm)**	**Cover (%)**	**Height (cm)**	**Notes**

Water Sampling

The water quality of river Ganga is important for the survival of aquatic organisms and can act as an indicator of health of the aquatic ecosystem. Thus, it is essential to monitor river water on regular basis in order to assess its health and suitability for survival of species of conservation significance. The present document provides guideline for step-by-step sampling procedure for collection of water, sediment, plant and tissue samples from river Ganga and associated quality control of samples.

1.0 Sampling procedure for collection of water samples

The present protocol provides the general and specific procedures, methods and considerations to be followed for the collection of Ganga river water. The surface water samples will be collected for quantification of chemical (organic and inorganic) contaminants including pesticides and heavy metals in order to determine overall toxicological profile of Ganga water.

1.1 Field Preparations And Collection

1.1.1 Sampling sites

There is no set number of sampling stations that can be considered sufficient to monitor all the possible types of waste discharges in mighty river Ganga. There is no routine methodology for site selection on a cook book basis. However, several factors will be considered while selecting sampling stations (e.g. flow, distance from urban localities,

presence of industrial belt, anthropogenic disturbances, conservation areas etc.) according to project objectives. GIS will be used to predetermine the sampling location that are representative of the matrix under investigation. The grab water samples of river Ganga will be collected using following steps-

After reaching to predefined sampling site, sampler will record all applicable information in the Eco-toxicological data log book for Water including; sampling personnel, weather conditions, site condition, sampling time, sampling method, etc. **(Appendix-2, Table 1)**

Research and scrutinize all proposed sampling locations to establish the accessible and representative locations.

Sampling will be conducted starting downstream working upstream to avoid disturbance of surface water quality.

Upstream/downstream of sampling point (dam/barrage) will be navigated to the actual sampling location for target environmental samples.

In unfavorable situations where access to river becomes extremely difficult or unsuitable then best available option would be selected w.r.t obtaining a sample from the chosen location.

Drinking water intake points, bathing ghats, irrigation canal off-take points will be selected to identify impact of such activities.

Upstream and downstream of all possible discharge outlets will be selected for sampling and additional downstream samples will be collected where mixing is poor and incomplete.

1.1.2 Sampling Times

Time of sample collection would be selected to cover all the seasons as the levels of many contaminants fluctuate over the year during different seasons. **1.2 Field Sampling Equipment**

Different scientific equipments are required to ensure that valid and accurate sample is obtained. The equipment checklist as given in **Appendix 1** and **Table 1** would be followed to ensure possession of the required supplies and equipment to ensure collection of decontaminated sample.

1.3 Water Collection Methodology:

The river water samples will be collected either directly filling the sample container or by decanting the water from a sample collection device.

Water samples for pesticides analysis will be collected in black/brown glass bottles cleaned with solvent used for extraction. Glass, teflon or aluminium extrusion containers are preferred.

Water samples for heavy metal analysis would be collected in a clean high density poly ethylene plastic or glass bottle.

Before collecting the final sample, the sample container will be rinsed three times with the sample.

Samples from shallow depths can readily be collected by submerging the sample container below 30 cm of water depth wearing proper gloves. With minimum surface disturbance, the sample bottle shall be submerged with the mouth of the container facing upstream and allowing sample stream to flow gently into the bottle.

A small air space in the bottle shall be left to allow mixing of sample at the time of analysis for water sample.

Water samples collected for heavy metal analysis need to fix at pH 2 using 0.5% HNO_3 whereas samples for heavy metal analysis can be stored without preservatives.

All the sample container will be labeled properly, by attaching an appropriately inscribed tag or label **(Appendix 4 Figure 4.1)**. The sample code and the sampling date would be clearly marked on the sample container or the tag.

Next, the sample identification form will be filled for each sample in Eco-toxicological sheet for water.

Samples will be collected from well-mixed section of the river (main stream).

The collected samples will be transported to the laboratory within 48 hr from the time of collection and will protect at 4°C temperature. This is because pesticide degradation is reduced at lower temperatures.

Sampling procedures will be done in same time periods (not more than two weeks) in order to compare between different sampling stations.

The collected samples will be tightly capped, adequately labeled and properly placed in sample container to allow easy transportation to laboratory.

Basic water parameters (Temperature, Dissolved Oxygen, pH, Conductivity, Total Dissolved Solids, Turbidity, and Hardness etc.) would be measured onsite through digital water probes as well as through traditional titrimetric method. All the onsite measured values would be filled in eco-toxicological data sheet **(Appendix 2, Table 2)**

1.4 Preserving and transporting samples

The sampler will be responsible for the transportation of collected samples to the laboratory in order to avoid any type of mishandling during transportation. After receiving at laboratory, samples will be stored in cool (-4^{o}C for pesticide) until further analysis.

1.5 Quality Control

Before leaving the field site, data sheets must be checked for completeness and readability. Data sheets will be checked by a different field crew member than the one who filled it out. That person marks each page “checked”, with their initials and the date.

The sampler will perform one calibration check analysis for pH, DO, and specific conductance using standard solutions prior to onsite recording at each stretch of study site.

The sampler will perform three replicate measurements to generate standard deviation in samples. The standard deviation of the three measurements must be less than 10 percent of the mean.

The seasonal sampling will be done on the same location to find out variability in concentration of parameters during different time periods.

Once on each field trip, sampler will fill one set of lab sample bottles with deionized water as field blanks.

II. SEDIMENT SAMPLING

The sediment sampling for the determination of chemical pollution in river Ganga is important as sediments acts as surface absorbent and natural filter for the various organic and inorganic chemicals present in water. Most of the borrowing aquatic organisms directly get in contact with deposited sediments and may get affected. Thepresent document provides preparations, appropriate methods and step-by-step instructions for the collection of Ganga river sediments and associated quality control of samples.

2.0 Sampling procedure for collection of sediment samples

The present protocol provides the general and specific procedures, methods and considerations to be followed for the collection of representative sediment samples from river Ganga. The sediment samples will be collected for quantification of chemical (organic and inorganic) contaminates including pesticides and heavy metals in order to determine toxicity, biological availability, extent and magnitude of contamination, contaminant migration pathway, potential source, fate of contaminants etc. in river Ganga.

2.1 Field Preparations And Collection

2.1.1 Sampling sites

Same as sec 1.1.1

After reaching to pre-determine sampling site, sampler will record all applicable information in the “eco-toxicological data sheet for sediments” **(Appendix 2, Table**

3) including; sampling personnel, weather conditions, site condition, sampling time, sampling method etc.

Sediment samples can be difficult to collect but are important in a residue sampling exercise. Water and sediment samples will be collected from same locations and at the same time to find a correlation between them.

2.1.2 Sampling Times

Same as sec. 1.1.2

2.2 Field Sampling Equipment

As per **Appendix 1, Table 2**

2.3 Sediment Collection Methodology:

Sediment will be collected from beneath an aqueous layer from the depth starting from 6-8 cm up to 10-15 cm either directly, using a hand held device such as a auger; or indirectly, using a remotely activated device such as dredge.

For sediment sampling one of the following techniques may be used:

(i) **Coring**: A PVC or Perspex tube (ca. 1 m x 8 cm φ) is used to extract relatively undisturbed sediment.

(ii) **Grabbing**: A larger volume of sediment, disturbed, however, can be collected. It is also useful for the collection of organisms.

(iii) **Others**: Special types of sediment samples have been developed, e.g. for use in sandy sediments (vibro-corers), for large sections of the sediment (box-corers).

New sampling bags would be used every time for collection of samples. Available guidelines suggest to fill up to ¾ parts of sampling bags and leaving small air space to allow mixing of sample at the time of analysis for sediment sample.

The sample container is to be labeled properly, preferably by attaching an appropriately inscribed tag or label. The sample code and the sampling date should be clearly marked on the sample container or the tag.

The sample identification form will be filled for each sample.

Samples will be collected from well-mixed section of the river (main stream).

The disturbing influences such as cattle wading, farming, fishing, and sand recovery can drastically influence chemical processes and the nature of the biological community. Such sampling sites will be avoided.

The collection of samples can be hazardous at some locations in bad weather (such as high flow). Such sampling sites can better be avoided.

Sampling procedures will be done in same time periods (not more than two weeks) in order to compare between different sampling stations.

All the freshly collected sediment samples should be store in fresh plastic zip bags of appropriate size and construction for the analyses requested.

The collected sediment samples will be representative of each sampling site and homogenized in nature.

Two different samples will be collected from each sampling site for heavy metal and pesticides analysis.

The sediment collecting sample bags will be tightly capped, adequately labeled **(Appendix 4, Figure 4.2)** and properly placed in sample container to allow easy transportation to laboratory.

Upstream and downstream of all possible discharge outlets will be selected for sampling and additional downstream samples will be collected where mixing is poor and incomplete.

2.4 Preserving and transporting samples

The sampler will be responsible for the transportation of collected samples to the laboratory in order to avoid any type of mishandling during transportation. After receiving at laboratory, the samples will be stored in cool and dry place until the further analysis.

III. FISH/TISSUE SAMPLING

3.0 SAMPLING PROCEDURE FOR COLLECTION FISH SAMPLES

3.1 Field Preparations And Collection

3.1.1 Sampling sites:

Same as sec 1.1.1.

3.1.2 Sampling time:

Same as sec. 1.1.2

3.2 Field Sampling Equipment

As per **Appendix 1, Table 3**

3.3 Fish Collection Methodology

3.3.1 Sample Size:

To enable a statistically sound data, the available guidelines suggest to collect up to 5 to 6 fish per species of nearly uniform size ranges. As a general guideline, the largest and smallest fish within each group should not exceed the average length of the group by more the 25%If the fish are small, more than 5 shall be collected so that there is enough tissue per sample for the lab analysis to be done. If there is an insufficient amount of tissue in each fish for analysis, then multiple fish and organ samples per site should be pooled to produce three composite samples per site

Tissue sample of at least 150-200gm is suggested for both toxicant types (i.e. inorganic/organics) as smaller amounts of tissue sample may lead to higher limits of reporting.

If dissection is not possible within 24 hours, whole fish may be frozen and its tissue sample can be taken prior to chemical analysis. However, extra consideration would be taken as excessive freezing may lead to rupture of internal organs.

For each fish sampled, the Eco-toxicologal data sheet **(Appendix 2, Table 4)** will be filled to record the species, length, weight, sample identification number, sapling location etc.

3.3.2 Collecting appropriate samples:

In order to obtain fresh tissue after a fish kill, the first preference is fresh fish then it is preferable to choose fish that are sick/ dying rather than dead (e.g. some might be moving but showing signs of lethargy or distress). If only dead fish are present (fish depot/market survey), choose the least decomposed fish available.

3.4 Fish Dissection: Place clean aluminum foil (for organics/pesticides analysis) or plastic (for metal analysis or other) on the dissection tray prior to placing the fish on the work area. Place a waste bag in an area easily accessible to the person conducting the dissections. Before embarking with specimen fixation, familiarize oneself with the general anatomy of the species taken up for investigation (**Appendix 3, Figure 3.1, 3.11**

& **3.12**). The whole procedure requires using powder-free gloves. Next the fish (±1 g) is weighed on a weigh balance. It is advisable to check zero and operational mode of balance between each fish, and re-adjusting or taring as necessary.

In the next step the length (cm) of the fish is measured on a decontaminated measuring board or by a decontaminated measuring scale **(Appendix 3, Figure 3.2)**. All the

physical attributes of fish sample like fins, skin and other external features are to be recorded on the eco-toxicological data sheet**.** Important conditions to note are deformities, scale loss, external parasites etc.

Muscle sample: The most accepted sampling protocol for muscle, gills and other internal organs is as follows:

Make a cut with the scalpel blade from just below the start of the dorsal fin down to the fish's lateral line (Cut 1, **Appendix 3, Figure 3.3**), then just above the lateral line of the fish toward the tail (Cut 2, **Appendix 3, Figure 3.3**), afterwards make a cut from where the first incision was made just below the dorsal fin across the top of the fish and down toward the tail (Cut 3, **Appendix 3, Figure 3.3**), to meet the cut from step 2. Remove the skin of this section of cut flesh using forceps and a scalpel blade (**Appendix 3, Figure 3.4**) and detach it from the small bones and allow it to be removed (**Appendix 3, Figure**

3.5). After taking muscle tissue take out other internal organs, as per study requirement. After each fish is dissected, all equipment should be cleaned and rinsed, and gloves should be disposed.

Gill samples: To collect the gills, lift the operculum or gill cover (**Appendix 3, Figure 3.6**), and make a cut at the base of operculum (**Appendix 3, Figure 3.7**). Carefully cut out the gills at their base (**Appendix 3, Figure 3.8**), taking care not to damage these when doing so. Rinse gills with de-ionized water. Place gills in labeled storage container/bag. In some larger fishes, gills may not require the operculum to be removed.

Other internal organ samples: Make a small cut just in front of the anus (vent) to open the abdominal cavity. With sharp-ended scissors, cut along the belly (ventral midline) of the fish, forward to the middle of the lower the jaw (**Appendix 3, Figure 3.9**).

Remove the flap of skin covering the abdominal cavity by cutting from the small cut in front of the anus upwards, across the body of the fish and toward the head of the fish (**Appendix 3, Figure 3.10**). This should expose the heart and abdominal organs for examination and removal.

Carefully cut out the organ for examination, taking care not to damage these when doing so. Rinse the removed organ with de-ionised water. Place the removed organ in labeled storage container/bag.

3.5 Preserving and packing samples: Once the muscle has been removed from the fish, rinse it in ultrapure water and place muscle/other internal organs sample in clearly labeled (**Appendix 4, Figure 4.3**), leak-proof containers. Place vial/container immediately in ice cooler/dry ice (24-48hr,-20°C) or liquid N_2 (>48hr,-190°C). Ensure that the sample vial/container (for keeping fish tissue) put inside liquid N_2 container are cryogenic. For heavy metals analysis, keep samples in acid washed plastic containers whereas for pesticide residue analysis, put samples in glass containers.

4 SAMPLING PROCEDURE FOR COLLECTION PLANTS SAMPLES 4.1 Field Preparations and Collections

Area of Sampling:

Same as sec. **1.1.1**

4.1.2 Field Sampling Equipment

As per **Appendix 1, Table 4**

4.2 Plant Collection Methodology

Sampling locations for plants/phytoplankton will be selected as near as possible to those selected for other sample types (water, sediment or fish), to ensure maximum correlation of findings and to enable a statistically sound data.

Sampling point would be established both upstream and downstream of a pollution source/dam/barrage or major tributary.

If practically feasible, then sampling points would also be set up on either side of the river, to account for unequal lateral mixing (in case of planktons).

As much of the plant sample as possible shall be collected from surface water plant as submerged plants.

Plants may be collected by hand pulling, standard pond net (wooden handle 1.5 m long) nets (for submerged plants) or using a weighted rake in deeper water.

All the physical attributes of plant sample will be recorded in the eco-toxicological data sheet **(Appendix 2, Table 6)** including the date, location, collector's name, and name of water body, flow rate, depth of water, substrate description (if plant is submersed, floating or emergent) color and odor, names of plant species associated with the collected plant.

The collected plant sample shall be washed with ultrapure or distilled water to remove algae, debris, and sediment.

4.3 Preserving and packing samples

The plant shall be wrapped in clean/new aluminum foil and placed inside a clearly labeled **(Appendix 4, Figure 4.4)** double zip locked bag.

APPENDIX 1

FIELD SAMPLING EQUIPMENT CHECKLIST- WATER SAMPLE COLLECTION

Table: 1

S.No	Equipments/Supplies	✓
1	Sampling site map	
2	Amber Glass bottles (1L)	
3	High density Poly ethylene bottles (1L)	
4	Onsite physico-chemical parameter measurement kit-YSI	
5	Life jacket & Rubber boots	
6	Note book, pen, pencil	
7	First-aid box	
8	Nitric Acid for preservation of samples (Heavy Metals)	
9	Torch, Spirit lamp, Match box, knife	
10	Tissue paper	
11	Extra heavy duty aluminum foil	
12	Disposable gloves (powder-free)	
13	Waterproof Permanent marker pen for labeling samples	
14	Teflon plastic squirt-bottles for dispensing acetone/ultrapure water/hexane	
15	Eco-toxicological datasheet/logbook	
16	GPS Unit for generating lat/longs	

17	Coolant material: dry ice or water (wet) ice	
18	Waste bags	
19	Cello tape (transparent)	
20	Parafilm (for sealing sample bottles)	

Table: 2

FIELD SAMPLING EQUIPMENT CHECKLIST- SEDIMENT SAMPLE COLLECTION

S.No	Equipments/Supplies	✓
1	Sampling site map	
2	Double Ziploc bags	
3	Life jacket & Rubber boots	
4	Note book, pen, pencil	
5	First-aid box	
6	Nitric Acid for preservation of samples (Heavy Metals)	
7	Tissue paper	
8	Extra heavy duty aluminum foil	
9	Disposable gloves (powder-free)	
10	Waterproof Permanent marker pen for labeling samples	
11	Teflon plastic squirt-bottles for dispensing acetone/ultrapure water/hexane	
12	Eco-toxicological datasheet/logbook	
13	GPS Unit for generating lat/longs	
14	Coolant material: dry ice or water (wet) ice	
15	Waste bags	
16	Cello tape (transparent)	
17	Parafilm (for sealing sample bottles)	

Table: 3

FIELD SAMPLING EQUIPMENT CHECKLIST- FISH SAMPLE COLLECTION

S.No	Equipments/Supplies	✓
1	Extra heavy duty aluminum foil	
2	Decontaminated and cleaned Scalpel (with sharp and disposal blades), Scissors and Forceps	
3	Fillet boards	
4	Disposable gloves (powder-free)	
5	Waterproof Permanent marker pen for labeling samples	
6	Measuring balance and scales (to weigh/measure fish prior to dissection)	
7	Teflon plastic squirt-bottles for dispensing acetone/ultrapure water/hexane	
8	Pesticide grade acetone/hexane for decontaminating knives	
9	Phosphate free liquid detergent for cleaning scissor, scalpels, and forceps	
10	Eco-toxicological datasheet/logbook	
11	GPS Unit for generating lat/longs	
12	Variety of sizes of zip closure plastic bags	
13	Coolers/ liquid N2 containers for cold storage of fish tissue samples	
14	Coolant material: dry ice or water (wet) ice	
15	Waste bags	
16	Cello tape (transparent)	

Table: 4

FIELD SAMPLING EQUIPMENT CHECKLIST- PLANTS SAMPLE COLLECTION

S.No	Equipments/Supplies	✓
1	Extra heavy duty aluminum foil	
2	Decontaminated and cleaned Scissors and Forceps	
4	Disposable gloves (powder-free)	
5	Waterproof Permanent marker pen for labeling samples	
6	Labels/Tags	
7	Teflon plastic squirt-bottles for dispensing acetone/ultrapure water	
8	Pesticide grade acetone/hexane for decontaminating knives	
9	Phosphate free liquid detergent for cleaning scissor and forceps	
10	Eco-toxicological datasheet/logbook	
11	GPS Unit for generating lat/longs	
12	Variety of sizes of zip closure plastic bags	
13	Rake/pond net	
14	Cello tape (transparent)	

APPENDIX 2

Table 1: Eco-toxicological data sheet for Water sample

Name of the study:

Type of Sample:

S.No	Date and Time of Sampling	Sample ID	Sample station and GPS coordinates	Sampling Depth	Distance from bank	Results of any measurements completed in field	Sample preservation & Storage	Other Observations/ Remarks

Table 2: Eco-toxicological data sheet for physico-chemical parameters of water

S.No	Date and Time of Sampling	Sample ID	River depth	River Width	Physico-Chemical Parameters					Other Observations/ Remarks
					pH	DO (mg/L)	TDS (S/m)	Temp (°C)	Turbidity (NTU)	

Table 3: Eco-toxicological data sheet for Sediment sample

Name of the study:

Type of Sample:

S.No	Date and Time of Sampling	Sample ID	Sample station and GPS coordinates	Sampling Depth	Distance from bank	Results of any measurements completed in field	Sample preservation & Storage	Other Observations/ Remarks

Table 4: Eco-toxicological data sheet for Fish sample

No	Date and Time of Sampling	Identification number	Sample station and GPS coordinates	Species & Type of tissue/organ collected	Length (cm)	Weight (gm)	Sex	Preservation	Any anomalies (e.g., lesions, cuts, sores, tumors, fin erosion) observed on the fish.	Remarks

Table 5: Eco-toxicological data sheet for Plant sample

S.No	Date and Time of Sampling	Sample ID	Sample station and GPS coordinates	Names of plant species	Depth of water	Water flow rate	color and odor of plant	substrate description (submersed, floating or emergent) and technique used to collect plant sample	Remarks

APPENDIX 3

General Anatomy of Fish for dissection purpose

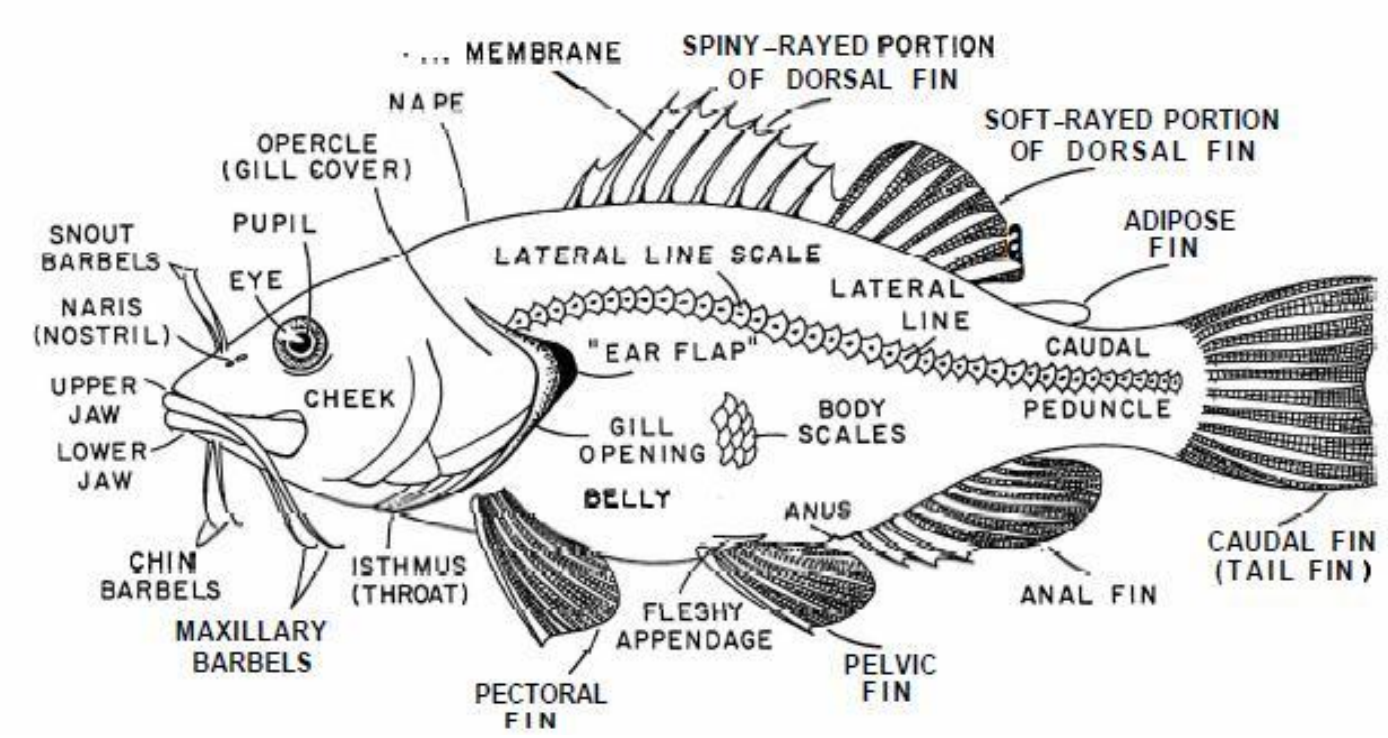
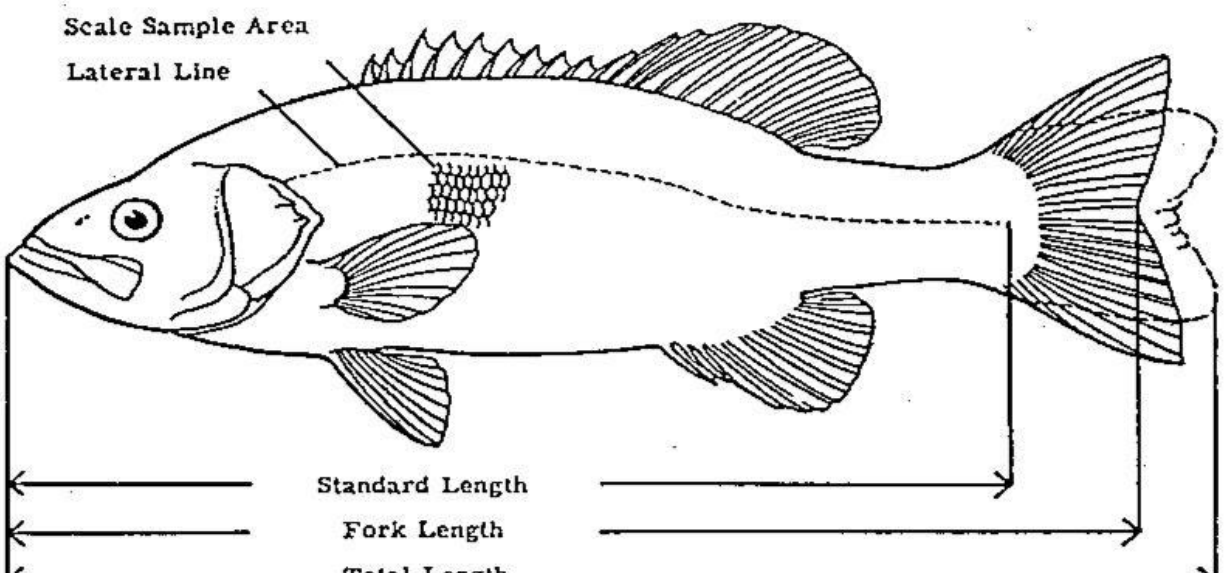


Figure 3.1 External features of a composite fish



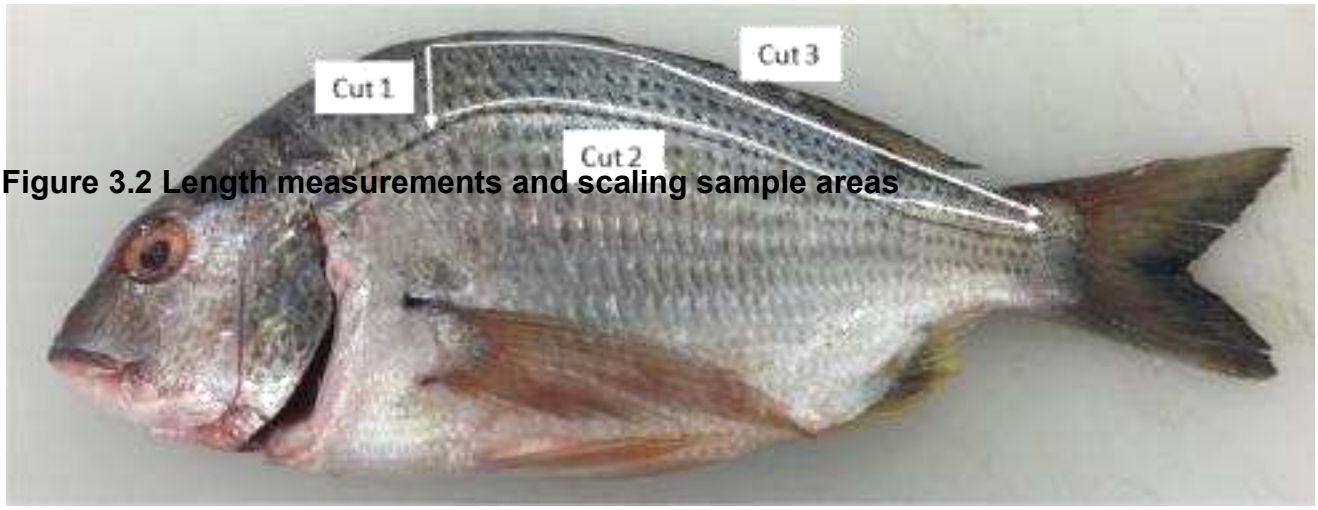


Figure 3.2 Length measurements and scaling sample areas

Figure 3.3 Outline of area to be removed from the fish for muscle sample*

Figure 3.4 Removing skin* **Figure 3.5 Removing muscle***

* *Environmental Protection (Water) Policy 2009 - Monitoring and Sampling Manual*

Figure 3.6 Lifting the operculum (gill cover) *

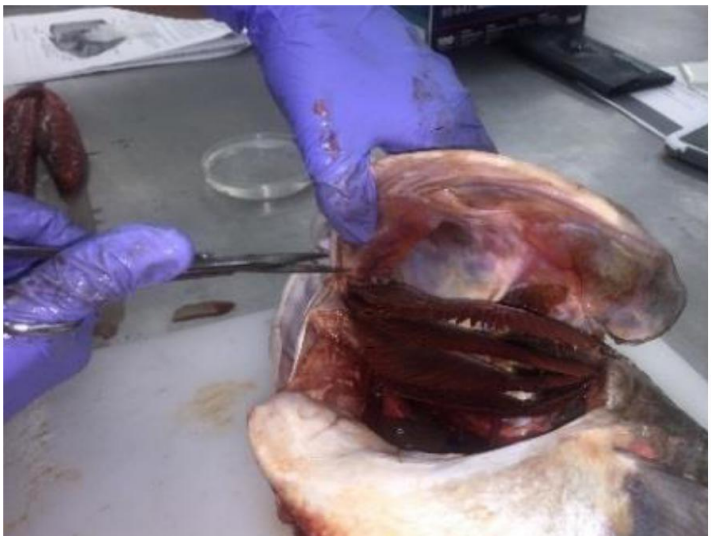


Figure 3.7 Cut at the base *

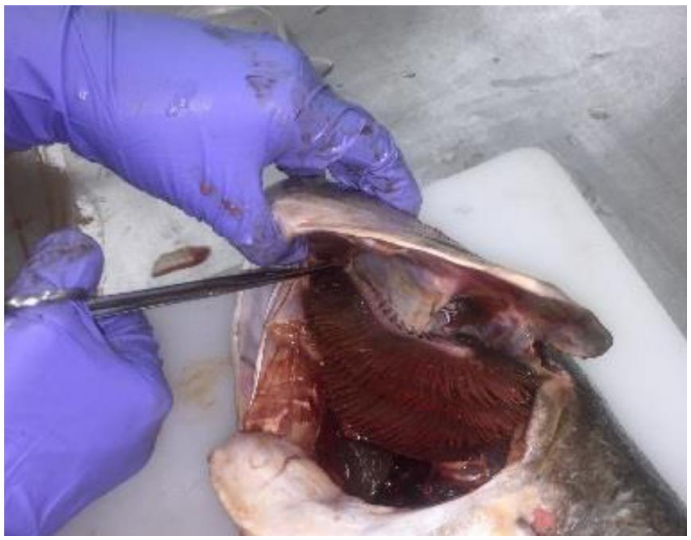


Figure 3.8 Cut gills at the base *

* *Environmental Protection (Water) Policy 2009 - Monitoring and Sampling Manual*

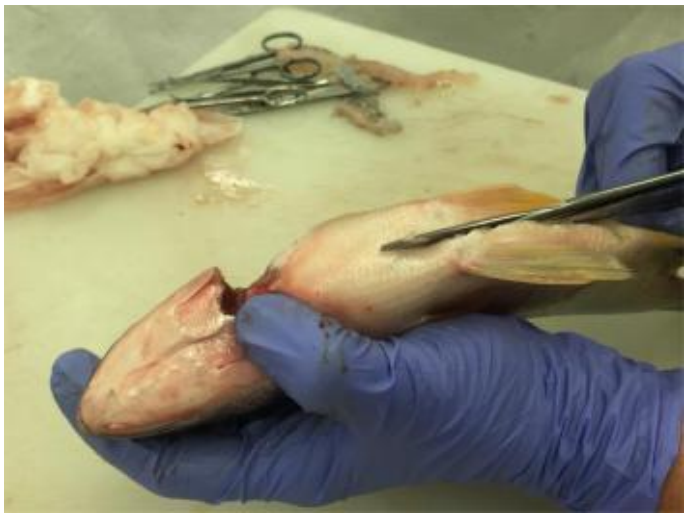


Figure 3.9 Cut along the belly *

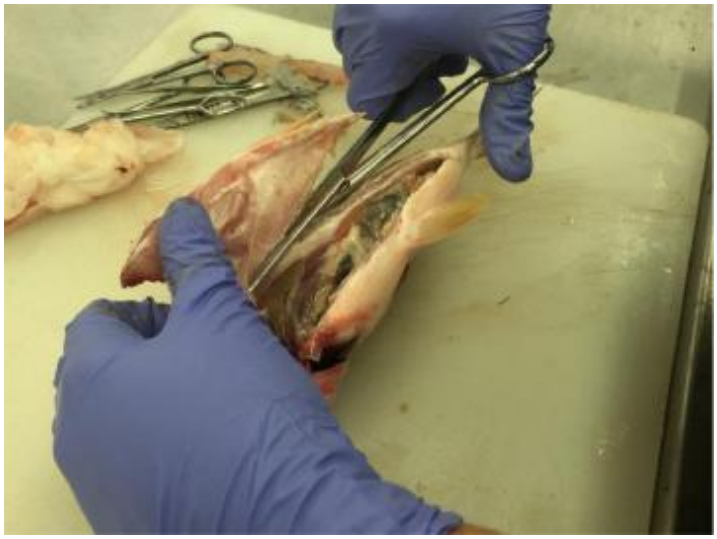


Figure 3.10: Expose the abdominal cavity *

* *Environmental Protection (Water) Policy 2009 - Monitoring and Sampling Manual*

Figure 3.11: Internal anatomy of a yellow fin bream*

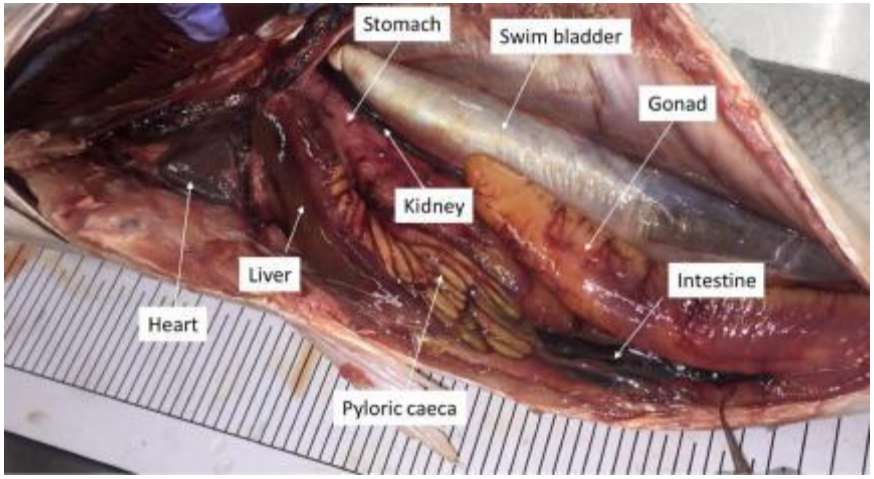


Figure 3.12: Internal anatomy of a dissected finfish

Note:Depending upon the body shape of the species, organs can be located in differing/varying places. The kidney is usually located up close to the spine and may be hidden by the swim bladder.

* *Environmental Protection (Water) Policy 2009 - Monitoring and Sampling Manual*

APPENDIX 4

Study Name :

Sample Code :

Sample Type /

Date & Time:

Sampling Location:

Collector:

Figure 4.1: Label to be used in sampling bottles (water samples)

Study Name :

Sample Code :

Sample Type /

Date & Time:

Sampling Location:

Collector:

Study Name :

Sample Code :

Sample/Species Type

Date & Time:

Sampling Location:

Collector:

Figure 4.3: Label to be used in sampling containers

(fish samples)

Figure 4.2: Label to be used in sampling bottles (sediment samples)

Study Name :

Sample Code :

Sample Type / Species :

Date & Time:

Sampling Location:

Collector:

Figure 4.4: Label to be used in sampling bags

(plants samples)

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