RAPID ASSESSMENT OF BIODIVERSITY

Brij Gopal

The term assessment is similar to evaluation or making judgment about the status of some object or activity of interest. It involves gathering and analysing qualitative and/or quantitative empirical data from many diverse sources on parameters of interest in order to understand the status of that object. It differs from Inventory which requires more extensive collection, collation and analysis of core (baseline) information on all components and aspects. It also differs from monitoring which necessarily involves collection of information at specific sites (locations) and at regular intervals for ascertaining any changes which may occur over time and to establish trends of change. Thus, the assessment of biodiversity involves collection and analysis of qualitative and/or quantitative information on the occurrence of various kinds of organisms in a defined area or habitat of interest, by actual field surveys.

PURPOSES OF BIODIVERSITY ASSESSMENT

A biodiversity assessment is required for many purposes. It has been assessed over past centuries simply for preparing catalogues of what exists in particular areas of geographical interest or in particular habitats. The habitats may be a pond, a lake, a certain river stretch, a forest, an agricultural field or a patch of grassland. The geographical area may extend from a village or town to a district, state or country or even a continent. Such assessments have often been made for only one kind of organisms; e.g., algae, invertebrates, fish, birds, flowering plants (or only aquatic macrophytes), and there too only one or more taxonomic group (such as blue-green algae, diatoms, rotifers, molluscs, or grasses). Geographical assessments sometimes include information on the broad habitat type, and qualitative information on abundance (rare or common). Estimates of density of constituent species are also made in recent studies but readily turned into indices of diversity, thereby losing useful information.

After it became clear that various anthropogenic activities adversely impact upon the biodiversity – and that the biodiversity is being lost rapidly, its assessment became an important component of environmental impact assessments (EIA) of developmental projects. The EIAs generally require a qualitative, or at best a semi-quantitative, documentation of the flora and fauna occurring in a specified area around the proposed site of the project, to provide adequate, reliable baseline data. Particular attention is paid to the rare, endangered and threatened species which may be adversely impacted by the project and hence remedial measures may be taken for their conservation. A post-project monitoring can help understand the impacts of the project over time though restoration will be extremely difficult.

Biodiversity assessments, particularly of a few groups of organisms, have been, however, of special interest in aquatic ecosystems because of the direct relationship between these organisms and the physico-chemical characteristics of water which they inhabit. Elaborate methodologies have been developed for the assessment and monitoring of water quality, and other habitat features using a fairly wide range of organisms – from diatoms and other algae (Whitton et al. 1991, Whitton and Rott 1996, Stevenson and Smol 2003), zooplankton (Jeppesen et al. 2011, Haberman and Haldna 2014), benthic macro-invertebrates (Rosenberg and Resh 1993, Freiberg et al. 2011), fish (Karr 1981, Simon 1999), amphibia (Welsh and Ollivier 1998) and even macrophytes (Wagner and Mikulyuk 2012). Obviously, an assessment of the diversity of these groups of organisms has attracted greater attention. A very detailed and comprehensive manual for field techniques and protocols of all taxa has been published by Eymann et al. (2010). More recently, Anderson and Davis (2013a,b) have provided detailed accounts of various methods for studying wetlands and their biota. Despite the focus entirely upon

North American wetlands and biota, the methods are certainly useful and applicable to most wetlands in the world and should be consulted.

RAPID ASSESSMENTS

It is extremely rare for the assessments of biodiversity for even a small habitat to cover all taxonomic groups from micro-organisms to mammals. Detailed and comprehensive assessments are constrained by several factors including the available expertise, time and resources. It is impossible for one individual to gain taxonomic expertise in many groups of and/or animals. Often, it is difficult to find an expert in all groups of invertebrates, or even the arthropods alone. Various biotic groups are then investigated by different experts at different times. Sampling of different groups of organisms requires widely divergent methods and effort. Identification of most of the organisms requires detailed morphological study of some organs or at some stage in their life cycle or a microscopic examination in the laboratory.

It is also not possible to make a fair assessment of the total biodiversity of even a small area for the simple reason that not all organisms will be found to be present together during one visit. Some are nocturnal, some live in burrows, some are seasonal migrants, and many have a short life span of few weeks or months. A few periodic visits (often monthly), by a team of several experts, are required during at least one year to assess the biodiversity of an area. Yet, the large inter-annual climatic variability – such as drought events – may affect the assessment seriously. The time required for a satisfactory assessment depends upon the purpose, the expertise, the resources, and the group of organisms and the level at which taxa (order, family, genus or species) are required to be identified.

To meet the variety of assessment needs, methods have been developed for rapid assessments of biodiversity. As the term implies, the assessment is made quickly over a shorter period that may still vary from a few days to a year. A rapid biodiversity assessment is not an exhaustive inventory and does not record every species in an area. Surveys over a longer period will certainly add more species to the list. It also depends upon the skills of the survey team and the methods used. Rapid biodiversity assessments report only the species which could be observed during the survey but do not show that which species are definitely absent. Certain groups of biota, particularly the bacteria, fungi and other microscopic organisms, are not amenable to rapid assessments and similarly, the genetic diversity cannot be assessed rapidly.

There are several publications on the rapid assessments of biodiversity of various inland aquatic ecosystems, including wetlands (e.g., Barbour et al. 1999). CBD and Ramsar Convention (2006) in their jointly published guidelines defined rapid assessment "*as a synoptic assessment, which is often undertaken as a matter of urgency, in the shortest timeframe possible to produce reliable and applicable results for its designed purpose*". They recognised five specific purposes of rapid assessment, somewhat similar to those described above:

1.Baseline inventory; prioritization; conservation; identification
2. Conservation of specific species; status of alien species
3. Change detection
4. Overall ecosystem health or condition, and
5. Sustainable use of biological resources

Accordingly, the types of Assessment were also categorised into five types corresponding to the purposes as:

1. Baseline inventory
2. Species-specific assessment
3. Change Assessment
4. Indicator assessment, and
5. Resource assessment

The baseline inventories include data on species lists, habitat types, some idea of population size and community structure and function, species interactions, abundances and distribution patterns, and important species (threatened, endangered, endemic, migratory, invasive aliens, and species of

cultural, scientific, economic social significance). In wetlands, information on hydrology and water quality also form part of baseline inventory.

Rapid assessments should be designed considering the objective, resources available, including time, money and expertise; scope, including taxonomic and geographic scope and site selection; sampling data and analysis, including level of identification of organisms, etc. It should be emphasised that the complex nature and variability of wetland ecosystems mean that there is no single rapid assessment method that can be applied to the wide range of wetland types and for the variety of different purposes for which assessments are undertaken. Rapid assessments of wetlands should be designed to take into account seasonal variability inherent in them, as many biota are seasonal migrants or utilise them seasonally.

Rapid Assessment of Biodiversity for Ecosystem Services Assessment

The present Guidelines have a limited objective of assessing those components of biodiversity which can be readily linked to different ecosystem services. As mentioned earlier, microorganisms play a significant role in many ecosystem functions of wetlands (especially nutrient cycles and carbon cycle related to green house gases) but their biodiversity or function cannot be assessed without detailed laboratory and field studies. Similarly, genetic diversity is of great importance from conservation viewpoint but cannot be assessed quickly and in the field. This version of guidelines is restricted to the methods of sampling and identification of major groups of organisms in inland freshwater wetlands, and among them also the peatlands and swamps (with woody vegetation) have not been included. Mangroves, salt marshes and coastal shallow waters with marine algae or seagrasses are outside the scope these guidelines. Notes are provided on the ecosystem services associated with the taxonomic group, and appropriate references are listed for identification of taxa to the level of family or genus, mostly from the South Asian region.

WETLAND DESCRIPTION AND HABITAT DIVERSITY

Before starting with the field survey for the biodiversity of a wetland, it is necessary to obtain basic information about the wetland itself as it will help in designing the sampling strategy and preparing for the field work. It will also be required for the assessment of several ecosystem services. This information may be available from earlier publications or may have to be collected by visiting the field and interacting with the local community.

Mapping of the wetland

A map of the wetland showing its location and geographic features of its catchment is the first most important requirement. This could often be adapted from the available survey maps or could be prepared from the remote sensing images such as those available from Google Earth. Maps with details of land use in the catchment may also be available from revenue records or other government offices. Features of geographical interest such as hills, streams, around the wetland should be also mapped. Further, human made structures, such as buildings, roads, plantations, crop fields, temples or recreational facilities and other features indicating human activities in and around the wetland should be mark ed on the map.

Basic features of the wetland

Following information should be obtained on the wetland:

● Total area of the wetland: This is determined at the maximum water level.
● Shape of the wetland area: Wetlands with irregular margin are likely to have considerably large area which undergoes large water level changes.
● Maximum and Mean depth: Maximum depth refers to the water depth at the time of highest water level and at the deepest point. The mean depth is computed from the maximum volume of water that can be held over the wetland and its area. It is NOT the arithmetic mean of a few water depth measurements.

● Bathymetric map: It is important to prepare a bathymetric map of the wetland and determine the area of the littoral zone (between the highest and lowest water level during the year), even if the wetland dries up completely during the dry season regularly or in some dry years.
● Nature of the Sediments.: The sediments which comprise of particulate mineral matter and organic matter, provide substrate for most of the wetland plants and many animals besides being the major source of nutrients for their growth. Sediments play a vital role in the nutrient dynamics of the wetlands and aquatic systems. Note down the nature of the substratum and sediments: whether it is rocky, gravelly, sandy or clayey; whether the sediments are soft or hard, and if there is a deposition of organic matter. The nature of the substratum and sediments may differ in different parts of the wetland.

Hydrology
In order to understand both the biodiversity and the ecosystem services, fairly detailed information is needed on the hydrology of the wetland. Besides obtaining information on the rainfall and temperature regimes of the area, examine the following:

Inflows: all sources of water, including surface runoff from the catchment. Does the water enter the wetland through drains/channels; if so, when (continuous flow, interrupted daily flow, seasonal flow); how much (assess the volume and its rate at different times, as necessary); and its quality (whether it carries domestic or industrial wastes, agricultural runoff or pollutants from other sources).
Outflows: Is there an outlet through which water is lost or withdrawn? Is it natural outflow or the water is abstracted by pumps or other means. Its amount and purpose as well as time of the year should be recorded.
Water level changes: Record the seasonal changes in water levels; and duration of water in the wetland at different depth contours.

Catchment and Land Use

Obtain information on different land uses within the catchment of the wetland paying particular attention to the activities which cause or may cause pollution in the wetland, and thereby may affect the biodiversity.

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PREPARATION FOR THE FIELD SURVEY

Before embarking upon a field survey, it is necessary to plan for it according to the field conditions and the nature of the studies to be made. In the first place, one should not go alone to the field. Adequate help is always required to carry the sampling equipment in the field, operating it for sample collection, preserving and carrying back the collected samples, recording observations in the field and in case of some unforeseen emergency. A team comprising of several persons is useful in collecting data on different groups of organisms at the same time and saves time and resources considerably. The team members should follow the following general precautions:

Use proper shoes. Waders should be used in shallow waters.
Life jacket should be worn, particularly when surveying with the boat.
Use hats, protective clothing (long sleeve shirts) and cream for protecting skin against UV radiation.
Take with you appropriate protection against sun and rain.
Protection against mosquitoes and leeches may also be required in tropical regions.

Ensure the following basic requirements, irrespective of the kind of organisms to be surveyed:

1. Map of the study area and the wetland
2. Field Protocol Sheets
3. Notebooks (preferably waterproof)
4. Permanent marker pens
5. Plastic bags, containers, Ziplock bags and waterproof labels
6. White plastic trays
7. Measuring tapes (2 m, 5 m and 30 m or more), and a rope
8. Magnifying hand lens
9. Binoculars
10. Camera
11. GPS
12. Identification keys and field guides
13. First aid kit
14. Ice box (for carrying samples).
15. Boat and life jacket (for non-wadeable waters)
16. Appropriate device to measure water depth
17. Plant cutter, and a knife
18. Rubber gloves
19. Portative Table (especially useful for sorting and processing the samples in the field)

RAPID ASSESSMENT OF BIODIVERSITY

MACROPHYTES

Brij Gopal

Macrophyte literally means large plant but the term is usually restricted in its use for macroscopic plants of water-dominated habitats in order to distinguish them from microphytes - the microscopic photyosynthetic organisms (uni- or multicellular algae).

In inland wetlands, macrophytes represent a large taxonomic spectrum of flowering plants, pteridophytes and bryophytes as well as algae. These plants occupy all positions within the water column and over the water saturated or submerged land. Some macrophytes float freely on the water surface with their roots (if any) hanging in the water, whereas some others remain suspended (whole plant body) in the water column. Vast majority of the macrophytes are rooted in or attached to the substratum. Their shoots remain either wholly submerged or emergent above the water surface. Some plants have their shoots creeping and floating over the water surface and yet others have only their leaves floating on the surface. Numerous macrophytes are rooted in waterlogged soils only. However, in majority of the flowering plants, the flowers are borne on or above the water surface (Sculthorpe 1967; Cook 1996).

The distribution of all macrophytes, except the free-floating ones, within a wetland is governed most by the water depth. Even the free-floating macrophytes have their best growth over shallow and calm waters away from the influence of waves and wind. Submerged macrophytes are restricted to shallow waters; the maximum depth of occurrence depends upon the penetration of light into water for photosynthesis (Caffrey et al. 2007) that is greatly affected by turbidity caused by the particulate matter including the phytoplankton. Submerged macrophytes without roots (e.g., *Ceratophyllum*) remain suspended nearer the water surface to exploit maximum light and may in turn reduce or eliminate light penetration to deeper layers. All rooted plants require sufficient light to sustain the growth of the leaves and/or shoots before reaching near, at or above the water surface. Further, the growth of emergent macrophytes is regulated by the water regime, i.e. the depth, duration and frequency and amplitude of water level changes. Differential response of the emergent macrophytes to water regimes results in zonation in distribution along the depth gradient within the littoral zone. Majority of the emergent macrophytes is restricted to a depth of less than 2 m. A large number of plants occur also on waterlogged or water-saturated substrates which may rarely experience submergence under thin layer of water. The morphology of such plants (e.g., species of *Ranunculus*) changes drastically with the water in and/or above the soil.

ECOSYSTEM SERVICES RELATED TO MACROPHYTES

Macrophytes hold the key to all kinds of ecosystem services of wetlands (Gopal 2015; Table 1). Majority of wetland fauna depends directly or indirectly on macrophytes as they are the major primary producers and support both the grazing and detritus food chains in food webs. Macrophytes are not only the food plants of many fish, birds and other herbivore fauna, many of them provide food for humans. Rice is the single most widely distributed macrophyte which has been domesticated and constitutes the staple food of more than half of the world's human population. Many other food plants include *Trapa bispinosa*, lotus, *Euryale ferox,* sedges such as *Eleocharis dulcis*, edible species of *Cyperus* and *Scirpus*, and *Ipomoea aquatica.* Jain et al. (2011) provide lists of plants from the Indo-Burma hotspot. Plants such as jute, *Aeschynomene*, reeds, cattails, and many grasses are used for thatch, ropes, mats and handicrafts, woody plants such as *Tamarix* sp. for fuel and several grasses and herbaceous macrophytes are important fodder or feed (e.g., species of *Echinochloa, Paspalum*, duckweeds). Many wetland plants have high medicinal value and have been traditionally used in South Asia (e.g., *Acorus calamus, Bacopa monieri, Hygrophila spinosa, Eclipta alba,* etc.).

Table 1. Ecosystem services of inland wetlands and deep open water systems (from Gopal 2015)

Ecosystem Services	Wetlands (mediated by macrophytes)	Deep open waters (Microphytes- dominated)
Provisioning Services		
Food	Many plants (especially rice), fish, amphibia, crustacea, molluscs	Only fish, mostly depend upon wetlands for their lifecycle
Fiber	Many plants, especially reeds, jute, cane, and tal grasses	None
Fodder	Many grasses and aquatic herbs	None
Fuel	Many plants	none
Biochemicals /Medicinal use	Numerous plants	A few algae
Regulating Services		
Hydrological regulation	Storage of water, flood regulation, altered water loss in evapotranspiration, groundwater recharge facilitated	Storage or transport of water, flood regulation, water loss in evaporation, variable groundwater recharge
Climate regulation-Carbon sequestration	Carbon sequestration; emission of GHGs (especially methane)	Extremely low carbon sequestration; methane emission from reservoirs
Micro-Climate regulation-	Temperature moderation	Temperature moderation
Erosion control	Yes, bank stabilisation; enhance sedimentation, prevent sediment resuspension	No
Water quality	Improve water quality in several ways	No; may be degraded by unchecked allochthonous inputs
Cultural/Recreational Services		
Recreation	Contribute to some kinds of recreation	Recreational activity specific to deep water
Landscape Aesthetics	Yes;	Yes, to some extent synergised by wetlands
Spiritual/Religious	Yes, location related; e.g. lotus in temple tanks	Yes, location specific; e.g. some high altitude lakes and river sources
Supporting Services		
Habitats for biota	Very high biodiversity	Few species
Soil formation	Yes, active contribution of plants	No
Pollination	Some wetland fauna involved	No

Macrophytes support biodiversity by providing habitats, food, nesting material and sites (Gopal and Masing 1990). Submerged macrophytes provide shelter for young fish and numerous macro-invertebrates (Bouchard et al. 2007, Thomaz and Da Cunha 2010). Numerous terrestrial biota (birds, insects and mammals) depend upon the macrophytes at one or the other stage of their life cycle for food and other resources (Batzer and Wissinger 1996, Winemiller 2004, Nhiwatiwa et al. 2009).

One of the most important ecosystem services mediated by macrophytes is to improve water clarity and quality as they absorb, accumulate and transform nutrients and a range of pollutants. Submerged macrophytes oxygenate the water column, lower the nutrient content and keep the water clear (Madsen et al. 2001). Most submerged plants oxygenate shallow water bodies (Nõges et al. 2003, Ahn et al. 2013). The rooted macrophytes actively transport oxygen to their rhizosphere in the sediments (Dacey 1980, Caraco et al. 2006) and thereby help mitigate the effects of anoxic conditions (Blute et al. 2004). Macrophytes check erosion, trap sediments, prevent resuspension of particulates, dampen the force of waves. Further, macrophytes play important role in the regulation of climate changes as they sequester carbon (Sahrawat 2003) as well as some of them serve as conduits for the

release of methane into the atmosphere from the sediments (Brix et al 2001, Laanbroek 2010). Macrophytes enhance biodiversity, support large populations of water fowl and enhance aesthetics. This in turn promotes recreation.

Fore more details of the functions and ecosystem services of wetland macrophytes, see Rejmankova (2011) and Gopal (2015).

METHODS FOR SURVEY AND SAMPLING

There are several methods for the survey and sampling of macrophytes depending upon the size, shape, depth, substratum and turbidity of water in the wetland. Wetlands which are small and wadeable, the entire area can be surveyed and sampled easily without requiring much sophisticated equipment. Deep water areas of small wetlands may be accessed by an inflatable rubber boat or a paddle boat. In large wetlands, a motor boat may be required to cover the distant areas, even if the water depth is 2-3 metres.

As mentioned earlier, different kinds of macrophytes occupy differen parts of a wetlands according to the water regime and generally exhibit a zonation along the water depth gradient from the margins toward he deeper areas (Figure 1). The deepest area of the wetland does not necessarily lie in its middle and the depth gradient often varies when examined from different points along the shoreline. In human-made wetlands such as reservoirs, the deepest part is generally close to the dam or bund. In irregularly shaped wetlands, much of the peripheral area covered by 'bays' is generally very shallow. It is therefore necessary that a quick survey of the wetland is made to understand different zones (which may vary in various parts of the wetland according to its depth profile) and that all zones are fully covered in the survey and sampling for macrophytes (as well as other biota).

Figure 1. Zonation of macrophytic vegetation in a wetland

Qualitative Assessment

The macrophytes occurring in the wetland can be readily collected and identified in the field by walking across it and wading into shallow waters. Free floating, floating leaved or submerged plants growing in an area that cannot be easily reached, can also be pulled out by using a rake with a long

handle. In case of wetlands with a large deep water area and where the macrophytes occur only in a relatively narrow littoral zone, the distribution of different taxa can be assessed visually and marked on the map.

Measurement of macrophyte abundance

The abundance of different species of macrophytes is important from the viewpoint of their relative contribution to the functioning of the wetland ecosystem and hence, their share in the ecosystem services as well as their economic value. An accurate estimate of the amounts of more important macrophytes is required for the assessment of ecosystem services, but a qualitative assessment must be made during the field survey. A 5 -point scale can be easily used to record the abundance based on visual estimation; for example, the abundance of each species may be scored as follows by combining observations on number of individuals (or area covered) and frequency of occurrence:

1 - Rare or very rare	Only a few individuals or a very small patch (as of duckweeds)	Very low frequency; observed at 1-2 places only
2 – Occasional	Several individuals or several patches (clumps)	Observed at a few places with similar water depth or waterlogging
3 – Common/Frequent	Many small patches or many individuals	Occur at several places with similar conditions
4 – Abundant	Many individuals or large patches	Occur in many parts of the wetland
5 – Very abundant	Form large stands, cover large areas of water surface	Occur almost every where

Several studies have recommended a 10 or 14-point scale based on cover, density and frequency but such details are generally required for monitoring or focused research on ecosystem functioning.

Quantitative Assessment

A systematic sampling strategy is required for quantitative assessment. The well known transect method of phytosociological analysis is most suitable for the study of macrophytes in wetlands which have almost invariably an environmental gradient of water depth. A one metre wide belt transect is more appropriate because several growth forms of aquatic plants often occur together.

A transect is laid perpendicular to the shoreline, and extending from the outer margin to the deeper area (Figure 2). The length of the transect depends upon the steepness of the slope and the depth up to which some submerged macrophytes occur in that wetland. In case of very large wetlands, it is not necessary to lay transects over the entire stretch where water depth does not vary significantly, and the composition of submerged macrophytes does not change. The number of transects depends upon the length of the shoreline, the area of the wetland and the variation in the distribution of macrophytes in different parts of the wetland. In any case, at least ten transects must be laid in a manner that they cover the entire variability in macrophytic vegetation and depth gradient.

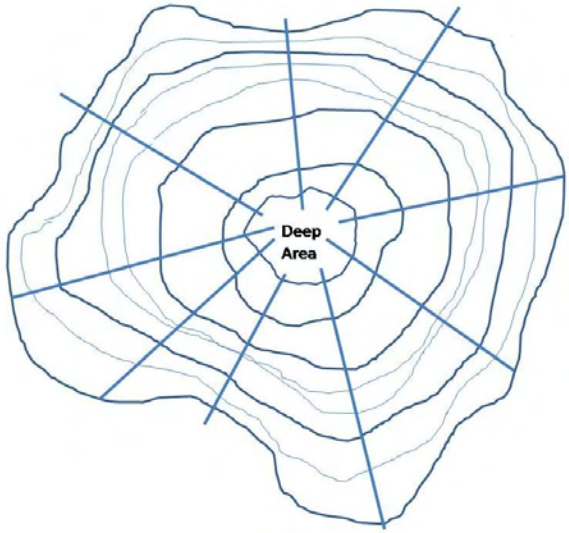

Figure 2. Plan for laying transects (thick lines) in a wetland. The depth contours are shown at 25 cm interval.

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Along the transect, samples are taken at such intervals that the effect of water depth of species composition can be examined. As far as possible, samples should be taken where the water depth increases by 25 cm at successive sampling point or at every 2 m or 5 m interval along the transect. As far as possible, 6-8 samples should be taken along each transect. However, this depends greatly upon the steepness of the slope and the width of the littoral zone.

Square quadrats of appropriate size (50 x 50cm or 1m x 1 m) should be sampled. The size can vary according the density and size of the macrophytes. In case of free floating plants like *Pistia, Salvinia* or *Spirodela,* it can be very time consuming to sample them in large size quadrats. A quadrat with a wooden frame can be used for free floating plants. For submerged and floating leaved plants in shallow waters, it is desirable to use a long square or circular cyclinder (open a both ends and made of acrylic sheet). It should be lowered in the water column and then the plans within it should be taken out with the help a rake (if manual collection is not possible). In deeper waters, and when the plants are not visible due to high turbidity, a rake or grapnel is used to sample the vegetation (Figure 3). Plants taken out of the water should be gently washed to remove soil and other debris adhering their roots and/or shoots. Submerged plants such as *Chara, Myriophyllum, Ceratophyllum, Najas, Zannichelia* are quite delicate. They should be handled with care and placed in water to avoid damage. Plants, particularly the submerged taxa, which have to be taken o the laboratory for proper identification should be placed in small amounts of water or preserved in 70% Ethanol with about 1% glycerol added to it.

Figure 3. A grapnel (left) and a rake (right)

Quantitative estimates of different species of interest can be made in terms of their areal coverage, volume or fresh or dry biomass. Because the free-floating macrophytes often occur in the same area of the wetland (or water column) as occupied by the submerged or emergent macrophytes, their relative values are confusing. Also, the drifting of the free floating macrophytes with the wind the often results in large variation in the density and cover within a short period that makes the correct estimation extremely difficult and unreliable. Extremely large differences in the growth form, size, volume and mass of different macrophytes makes it necessary to estimate the densities or biomass of species of each growth form separately. Large amount of water content of macrophytes that may exceed 98% of the fresh biomass also means that the quantitative estimates based on fresh weight have practically little value. The dry biomass of different macrophytes is estimated best by harvesting all plants from a predetermined area (10 x 10 cm area for small free floating plants, 25 x 25 cm area for submerged plants and herbaceous plants on waterlogged soils, and 50 x 50 cm for emergent and floating leaved macrophytes). The plants are carefully washed, blotted free of adhering water (without pressing hard), dried in hot air oven and weighed. The biomass is expressed as g m^{-2} or kg ha^{-1}.

IDENTIFICATION OF WETLAND PLANTS

There are very few publications to help in the identification of macrophytes in the wetlands of South Asian region. Biswas and Calder (1937) and Subrahmanyam (1962) described the more widely distributed plants along with their illustrations. Cook (1996) published a comprehensive and detailed account of all wetland plants occurring at altitudes below 1000 m. Macrophytes occurring above that elevation (mostly in the Himalaya) are generally common taxa in the temperate European climate. Identification of the macrophytes at the species level, particularly in the case of grasses and sedges, and the genera with many species, will require laboratory examination of reproductive parts (flowers). Given below is a simple key to a few common taxa that can be identified to the genus level in the field. The growth form of the macrophytes is generally a good starting point although some genera such as *Potamogeton* include species with both submerged and floating leaved forms and exhibit large morphological diversity among their species.

Free Floating Macrophytes

A. Plant body frond-like; shield shaped, flattened; roots present
Duckweeds **(***Lemna*, *Spirodela*),
B. Plant body globose, without roots. *Wolffia*
C. .Plants with sessile leaves
a. Floating leaves opposite, folded, upper surface with hairs *Salvinia*
b. Floating leaves alternate, minute, upper surface without hairs *Azolla*
c. Leaves forming a rosette (whorl like) *Pistia*
D. Plants with petiolate leaves
a. Petioles long swollen or globose, lamina orbicular *Eichhornia crassipes*


(Left) *Salvinia molesta and (Right)Spirodela polyrhiza* (small fronds), *Wolffia arhiza* (small dot like) and *Nymphoides* sp. (large leaf and white flowers)


(Left) *Pistia stratiotes* and (Right) *Eichhornia crassipes*

Submerged Macrophytes

A. All leaves emerge from same place - Plants with a rhizome or stolon

a. Leaves long, narrow, bear spores at their base	*Isoetes* (a pteridophyte)
b. Leaves long ribbon like	*Vallisneria*

B. Leaves arranged along the stem

a. Leaves simple, entire, flat, undivided	
Leaves in whorls of three or more at each node	*Hydrilla, Najas*
Leaves opposite or alternate	*Callitriche, Potamogeton*
b. Leaves finely divided, or narrow rounded	*Myriophyllum, Utricularia, Ceratophyllum, Chara, Nitella*

Macrophytes with leaves floating on the water surface

A. Leaves without a jointed sheath, peltate, entire, orbicular	*Nelumbo, Nymphaea, Euryale ferox, Nymphoides*
B. Leaves triangular, swollen petiole, saw-tooth margins; form a rosette	*Trapa*
C. Leaves four-lobed, long petiole	*Marsilea*
D. Leaves elliptic, petiolate	*Potamogeton natans, Aponogeton*

Macrophytes with shoots creeping on the water surface

A. Leaves shining surface, ovate, entire margin	*Ludwigia (=Jussiaea*)
B. Leaves simple, triangular, petiolate	*Ipomoea*
C. Leaves compound, bipinnate, stem often swollen and white	*Neptunia*

(Left) *Nelumbo nucifera,* (Right) *Marsilea minuta*

(Left) *Ipomoea aquatic* (Right) *Potamogeton natans*

Emergents

Most common taxa are species of : *Typha, Phragmites, Acorus, Arundo, Carex, Eleocharis, Cyperus, Scirpus, Sparganium, Sagittaria, Aeschynomese, Polygonum, Paspalum, Echinochloa, Hygrorhiza, Momochoria, etc.*

Identification in vegetative state is often very difficult unless one knows the plant already.
Follow taxonomic keys mentioned earlier. Grasses are still difficult to identify without microscopic examination of flowering parts.

Macrophytes on Waterlogged Substrates

A very large number of herbaceous macrophytes grow on moist to waterlogged soils. Like many other macrophytes (such as water hyacinth, Marsilea), most of them exhibit extreme morphological plasticity making identification difficult. They may also grow as emergents in shallow waters. Most often, identification to the species level requires flowering individuals. Some of the more common macrophytes of waterlogged soils, occurring in peripheral areas of wetlands, and especially during the drying phase are: *Eclipta alba, Alternanthera sessilis and A. philoxeroides, Bacopa monnieri, Ran*unculus sp., *Equisetum* sp., *Hygrophila* sp., etc.

Eleocharis sp.

Hygrophila spinosa

Cyperus sp.

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RAPID ASSESSMENT OF BIODIVERSITY

MICROPHYTES (PHYTOPLANKON AND PERIPHYTON)

Suman Kumari and **M.K. Bandyopadhyay**

Microphytes are microscopic plants, nearly always comprising of algae. Algae are a large and diverse group of simple, autotrophic (photosynthetic) organisms. They are dominant in aquatic habitats though many of them occur in dry environments. Morpholgically they may be unicellular, colonial or filamentous. They may be planktonic or attached to substrates. In freshwater ecosystem, algae occur either free floating (planktonic) or associated with bottom substrates (benthic algae). Planktonic algae dominate mainly in lentic waters (lakes, wetlands) with seasonal variation. Benthic algae occur in the bottom of shallow lakes, wetlands and rivers and are associated with sediments, rocks and organic debris.

Plankton are microscopic or minute organisms suspended in water column (along with some plants and detritus) whose distribution is influenced by wind, current and tides. However, the term has been limited in usage to two major groups, namely, phyto-plankton (photosynthetic organisms, chiefly algae) and zooplankton (planktonic animals). Plankton vary greatly in size. Some phytoplankton, protozoa and bacteria are less than 10 μm and pass through the finest plankton net, which are called *nannoplankton.* The larger forms which are retained by standard plankton nets are designated as *net plankton* or *filterable plankton*. The generally accepted classification of plankton based on sizes are Ultra nannoplankton (below2μm), Nannoplankton (2-20 μm), Microplankton (20-200 μm), Mesoplankton (200-2000 μm) and Megaplankton (above 2000 μm).

Generally water temperature, current and wind action control the distribution of plankton in a water body. It is necessary to have representative samples for proper assessment of plankton in an aquatic ecosystem.

PHYTOPLANKTON

Phytoplankton comprise of algal groups, chiefly Chlorophyceae (green algae), Cyanophyceae (blue green algae) and Bacillariophyceae (diatoms). The phytoplankton form a vital part of the aquatic food web and therefore information on their populations is often important to wetland management. Methods for their qualitative and quantitative studies are described in several standard books (e.g., Wetzel and Likens 2000, Wehr and Sigee 2015).

Phytoplankton are usually categorized according to size (Table 1):

Table 1. Different categories of phytoplankton.

Category	Size range (μm)	Common examples
Picophytoplankton	0.2-2.0	Photosynthetic bacteria, Blue green algae; *Synecococcus, Synecocystis Aphanothece, , Aphanocapsa*
Nanophytoplankton	2-20	Blue green algae; *Chroococcus, Merismopedia* Green algae; *Scenedesmus, Crucigenia, Cryptomonas, Rhodomonas* Bacillariophytes; *Fragillaria, Cyclotella*
Microphytoplankton	20-200	Dinoflagellates; *Ceratium, Peridinium,* Bacillariophtes: *Pinnularia, Cymbella, Asterionella*
Macrophytoplankton	>200	Green algae: *Volvox, Pandorina, Eudorina* Blue green algae; *Anabaena, Microcystis, Nostoc*

The systematic classification of fresh water algae is modified by Robert Edward Lee, 2008 and these algae classified into four distinct group:

Prokaryotic algae: In these algae membrane-bound plastids, endoplasmic reticulum, mitochondria, golgibody and large aqueous vacuoles are absent; nuclear materials (deoxyribo-nucleic acid) is dispersed throughout the cells.

Eukaryotic algae: the cells of eukaryotic algae have localized DNA similar to higher plants, membrane bound cell organelles such as plastids, Chloroplast, endoplasmic reticulum, mitochondria, golgibody and large aqueous vacuoles are present.

- Group1. Prokaryotic algae: Blue green algae (Cyanophyta/ Cyanobacteria): Cell division amictic and process called fission.
  Pigments: Chlorophyll a associated with thylakoids; Phycobiliprotein
- Group 2. Eukaryotic algae with chloroplast surrounded by double membranes of the chloroplast envelop; Chlorophyta (Green algae)
  Pigments: Chlorophyll *a* and *b*, Carotene and Xanthophylls
- Group 3. Eukaryotic algae with chloroplasts surrounded by one membrane of chloroplast endoplasmic reticulum; Euglenophyta (*Euglena*, *Phacus* and *Trachelomonas*)
  Pigment: Chlorophyll *a* and *b*
  Dinophyta (Dinoflagellates): *Ceratium* and *Peridinium*
  Pigment: Chlorophyll *a* and *c*
- Group 4: Eukaryotic algae with chloroplast surrounded by double membranes of chloroplast endoplasmic reticulum; Bacillariophyta (Diatoms): Pennate and centric diatoms
  Pigments: Chlorophyll *a*, c_1 and c_2, yellowish green or brown (fucoxanthin) with carotinoids predominant
  Xanthophyta (Yellow-green algae): *Tribonema, Botrydium, Vaucheria*
  Pigments: Chlorophyll *a* and *c*, β-Carotene, Xanthophyll

Table 2. Characteristic features of different algal classes or groups.

Algal class	Common name	Colour	Flagella	Nucleus	Structure	Planktonic /Benthic	Examples
Cyanophyta	Blue-green algae	Blue-green	Absent	Absent	Unicellular	Planktonic	*Synechococcus, Aphanothece, Aphanocapsa*
					Colonial	Planktonic Benthic both	*Microcystis, Anabaena, Merismopedia, Oscillatoria, Gloeotrichia,*
Chlorophyta	Green algae	Green	Present Absent	Present	Unicellular	Planktonic	*Chlamydomonas, Rhodomonas*
					Colonial	Benthic	*Pandorina, Volvox, Eudorina, Gonium*
					Filamentous	both attached and can become planktonic	*Microspora, Zygema, Spirogyra, Oedogonium, Cladophora*

Xanthophyta	Yellow-green algae	Yellow-green	Present (in two unequal length)	Present	Unicellular Colonial Filamentous	Planktonic/benthic Planktonic	*Botrydium, Botrydiopsis, Characiopsis Gloeobotrys Ophiocytium Tribonema, Heterococcus*

Table 2. Characteristic features of different algal classes or groups (continued).

Algal Class	Common name	Colour	Flegella	Nucleus	Structure	Planktonic /Benthic	Examples
Bacillariophyta	Diatoms	Brown light green	True flagella Absent	Present	Centric	Planktonic	*Cyclotella, Stephanodiscus*
					Pennate	Planktonic / Benthic	*Aulecosira* *Pinnularia, Navicula, Synedra*
Dinophyta	Dino-flagellates	Brownish	two terminal flagella of different morphology	Present	Unicellular	Planktonic	*Ceratium, Peridinium*
Euglenophyta	Flagellates	Green and brown	Two equal or unequal anterior flagella present.	Present	Unicellular	Planktonic	*Euglena, Phacus* and *Trachelomonas*
Charophyta	Desmids	Green and Brown	Absent	Present	Unicellular	Planktonic	*Selenastrum, Micrasterias, Closterium*
					Colonial	Benthic	*Coelastrum, Pediastrum*
Bacillariophyta	Diatoms	Brown light green	True flagella Absent	Present	Centric	Planktonic	*Cyclotella, Stephanodiscus*
					Pinnate	Planktonic / Benthic	*Aulecosira,* *Pinnularia, Navicula, Synedra, Nitzschia*

Plankton Sampling Strategy

Sampling strategy for phytoplankton varies according to several factors. Some are noted below:

- Morphology and hydrology of water bodies: depth of water, size of the water body, presence of macrophytes, flow, etc.,
- Strategy for phytoplankton sampling varies for lakes, reservoirs and wetlands from that for rivers and estuaries)
- Sampling frequency and time within seasonal cycle depends on the objective of the study
- Selection of sampling sites also depends upon the physical features; standing or flowing, inflows or outflows, pollution sources and kinds, otherhuman activities in or around the water body/wetland).
- In rivers, due to vertical and horizontal mixing of water, collect phytoplankton samples at midstream 0.5 to 1metre below the surface.

- In a lake or reservoir, a grid network or transects are used in combination with random procedures to take a sufficient number of samples.
- Where water is deeper than 1 meter, phytoplankton populations should be sampled from several depth zones (0.5 or 1.0 m interval).
- Label the sample containers with date and time, sampling station, study area (river, lake, and reservoir), type of sample and depth.

Phytoplankton Collection Methods

(A) Net method.

The easiest way to obtain a concentrated plankton sample is to tow a cone-shaped net (bolting silk or monofilament nylon) through the water. The wider end of the net is kept open by a metal hoop and attached to the tow rope by a bridle. The narrow end is closed by a metal or plastic receiving vessel. When towed through the water, a back pressure builds up at the opening which prevents some water flowing through the net. A tapering canvas sleeve allows more effective filtering by reducing the volume of water entering the net. A coarse net ensures a fast flow suitable for collecting larger zooplankton. Slow filtration occurs with nets of finer mesh sizes.

Net samples can be collected from various depths and vertical hauls made between levels with the aid of a throttling device around the canvas opening. The throttling device is triggered by a metal weight or messenger sent down the cable from the boat. Flow meters give more accurate information on the quantities of water flowing through the net. For good filtration, the ratio of the filtering area of the net to orifice area should be at least 3:1. Three types of tows are used- vertical, horizontal and oblique.

Vertical tows- These are preferred to obtain an integrated water column sample. To make a vertical tow, lower the weighted net to a given depth, then raise vertically at an even speed of 0.5m/s.

Horizontal tows- These are usually used to obtain depth distribution information on zooplankton. For horizontal towing, from a boat lower the net to the preselected depth, tow for 5-10 minutes and then raise it.

Oblique tows- These are preferred over vertical tows in shallow water or wherever a longer net tow is required. For oblique towing, lower the net to the prefixed depth and then rise at a constant rate as the boat moves forward.

In the completely enclosed environment, plankton samples are generally collected by using truncated cone shaped net made of bolting silk (No.25; mesh: 0.064mm). The upper circumference (30cm) of the net is attached to a brass ring with a handle and the lower narrow circumference (9.2cm) is fixed to the mouth of a collecting plastic or glass tube. A known volume of water (50 or 100 litres) is collected from the selected sites/directions/depths and then filtered through the net to obtain plankton samples.

(B) Tube and water bottle method

In calm condition, a length of hosepipe (5m length) weighted at one end is lowered into the water to enclose a known volume of sample. With closed upper end, the lower (weighted) end of the pipe is hauled by means of an attached cord and the water sample with plankton is transferred to a clean container.

Secondly, a weighted glass or plastic bottle of known capacity and sealed with a rubber bung can be lowered to a required depth in the water. The bung is fixed to a length of stout line and is removed at the required depth.

Fixed volume of plankton samples are also collected from deeper waters with more sophisticated sampler namely Kemmerer sampler, Van Dorn sampler, Niskin sampler and Nansen sampler. The commonly used Van Dron sampler is an open cylinder of known capacity that is let down into the water and automatically closed at both ends by a metal weight or ‘messenger’ which

slides down the cable. The enclosed water is under pressure so that more water cannot enter at other levels during passage to the surface.

(C) Suction pump method.
A suction pump with a weighted tube of required length can be used to collect plankton organisms at successive levels throughout a water column. The pumps help in collection of large samples with greater speed and accurate quantity. Diaphragm or peristaltic pumps are less damaging to the organisms than the centrifugal pump. Through pump a homogeneous sample from a given depth or an integrated sample from the surface to a particular depth is obtained.

Preservation of sample.

For preservation 4% to 5% formaldehyde solution is added to the collected plankton sample. However, depending on the purpose of sampling and investigation several fixatives are also used as follows:

• **Lugol's solution**- It is the most suitable phytoplankton preservative. Lugol's solution is prepared by dissolving 20g potassium iodide (KI) and 10g iodine crystals in 200ml distilled water containing 20 ml glacial acetic acid. For short term storage add 0.3 ml Lugol's solution to 100 ml sample and buffered formaldehyde to a minimum of 2.5% final concentration after one hour.

• **Formalin**- To preserve samples with formalin, add 40 ml buffered formalin (20g sodium borate + 1 lit of 37% formaldehyde) to 1 lit of sample immediately after collection.

• **"M3" fixative**- Dissolve 5g potassium iodide (KI), 10g iodine, 50ml glacial acetic acid and 250ml formalin in 1lit distilled water to prepare M3 fixative. Add 20 ml M3 fixative to 1 litre sample and store in dark place.

• **Other preservatives**- 95% alcohol and 6-3-1 preservative (6 parts water, 3parts 95% alcohol and 1 part formalin). Use equal volume of preservative and sample.

Qualitative and Quantitative Assessment of Phytoplankton

An assessment of phytoplankton invariably requires concentration of the organisms in the water sample through flitration, centrifugation or sedimentation. Filtration through plankton nets is often used to collect the samples as described above but has the disadvantage because either the fine-mesh nets are quickly clogged or the small sized plankton escape collection, and result in incorrect estimates of actual kinds of plankton and their densities. Centrifugation distorts the shape and size of the cells, packs the cells closely making removal difficult from the centrifugation tubes. At low velocity of centrifugation, nanoplankton are likely to remain in the supernatamt. The sedimentation method requires the samples to stand for long time to settle down according to density and size. The only disadvantage of sedimentation method is the long time required (usually 24 hours) for the samples to available for microscopic examination.

For counting a variety of counting cells are used of which the Sedgewick Rafter Cell and Haemocytometer are quite common. Whereas the haemocytometers do not allow larger cells or colonies of fragments of filaments to be correctly represented, the Sedgewick-Rafter cell makes the mall plankton (micro- and nano) difficult to be estimated. Braarud (1958) stated that "*net methods should not be employed for quantitative phytoplankton studies*". The simplest and most effective and accurate method is by sedimentation of the phytoplankton after preservation. The best method of choice is to use the Utermohl's Sedimentation Chambers. There are several designs now available but all of them combines the sedimentation on to a slide or cover slip thatcan be examined under a regular light microscope or inverted microscope.

i) Utermohl's Sedimentation chambers

Depending upon the expected density of phytoplankton, 5 ml to 100 ml of the preserved water sample is taken, after thorough homogenisation, in the Utermohl's sedimentation chamber of that size (Figure). After filling the chamber, it is covered with a glass plate and placed at room temperature, away from direct sunlight and on a flat surface without ny vibration or disturbance. The sedimentation chamber should not be gripped by hand to avoid any slight increase in temperature. After 24 hours, the supernatant is drained out carefully with a pipette or syringe without disturbing the sedimented plankton. It can then be examined under an inverted-microscope. Cell counting should be made on the whole counting chamber for the less abundant species and on diameter transects or random fields for the dominant species. Cell abundance is computed from the number of cells on the observed surface and its ratio to the total surface area of base plate of the chamber.

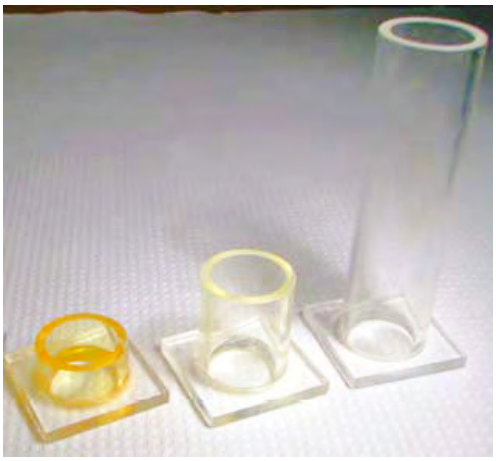

Figure 1. Utermohl's sedimentation chambers

i) Sedgewick-Rafter (S-R) counting cell method

The dimension of S-R cell is 50mm x 20mm x 1mm with total volume of 1000 mm^3 or 1ml. Transfer well shaken 1ml phytoplankton concentrate in the S-R cell, place a cover glass and put under the microscope for counting. Frequencies of different phytoplankton species are noted at random from each of randomly selected 10 squares out of 1000 squares of the S-R cell and the average of these are used for estimation.

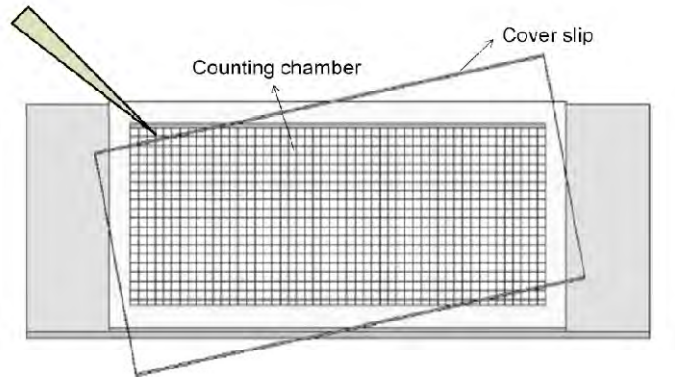

Figure 2: Sedgwick rafter counting cell

If ni is the number of a species occurring in a square of the counting cell then the number 'N' for the species in the total water volume filtered (whole sample) is calculated as;

$$N = \frac{n1 + n2 + n3 + n4 + n5 + n6 + \ldots\ldots + n10}{10} \times 1000 \times P\,C\ (ml).$$

[Where, PC = Phytoplankton Concentrate.]

The total units (numbers) of 'N' present in 1 litre of original water are estimated as follows:

$$u/l = \frac{N}{\text{Litres of water filtered}}$$

ii) Quick Drop Method

The drop method is used for quick analysis of phytoplankton. First the concentrated phytoplankton is diluted to 5ml or 10 ml as convenient, mix well and then a known volume of sample using micropipette drop is drawn (for example 50µl of each drop; 20 drop make up to 1ml). Then the drop is put in a glass slide covered with a cover slip and placed under the microscope for counting. All the phytoplankton species present under the cover slip covered area are counted and their respective frequencies are recorded. Likewise, few drops are taken and the organisms are counted. The number 'n' for any particular species is taken as the mean value of that species in the total counted drops.
Now, the total number of that species present in 1litre of original water is calculated as follows:

$$u/l = \frac{n \times 20 \times \text{Phytoplankton concentrate (5 or 10) in ml.}}{\text{Volume of water filtered (litres)}}$$

iii) Haemocytometer cell counting method

- Haemocytometer is a sturdy slide designed to hold a suspension of cells in a thin layer over a graticule etched on the slide (Figures 3-4).
- A group of 25 large squares in the centre are each further divided into 16 small squares.
- The middle section of the slide is lower than the outer platform. The cover slip is thick so that it resists bending.
- Use a capillary pipette or a syringe to introduce sample below the cover slip.

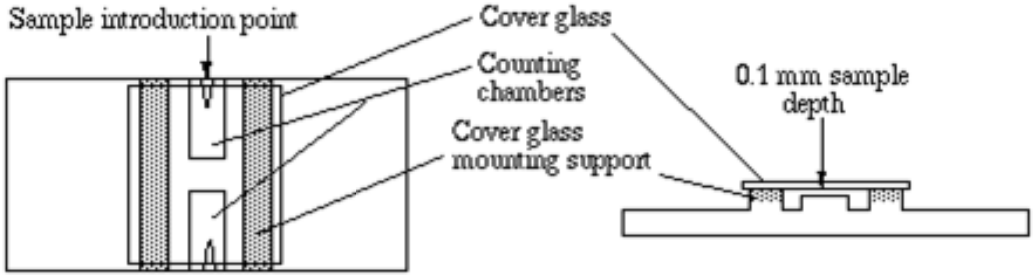

Figure 3: Diagram of the parts of the haemocytometer (Source: Team: Concordia/Notebook/2014)

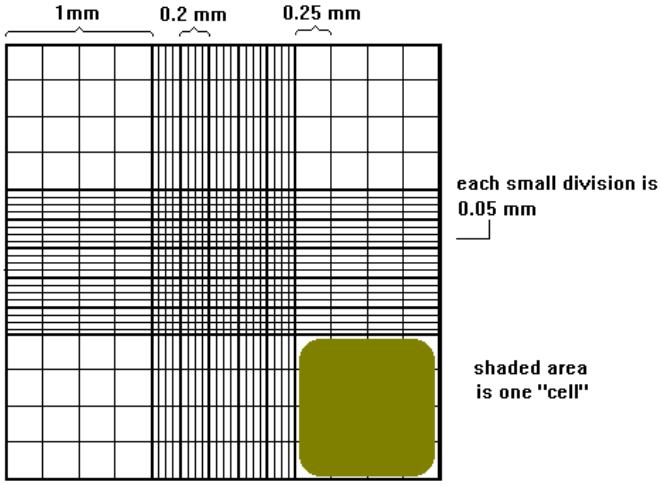

Figure 4. Haemocytometer counting area (Source: http://www.deltaenvironmental.com.au/)

Measurement of Plankton Biomass

The biomass of collected plankton samples can be measured through any of the following methods.

(A) Wet weight method.

Remove water from the collected plankton sample using a filter paper.
Blot out water as much as possible from the sample.
Weigh the sample and record plankton biomass in gram.

(B) Volumetric method.

It can be done either by settlement or displacement method.

To measure by settlement method, place the plankton sample in a narrow measuring cylinder and allow standing for one day for plankton settlement. Note and record the volume occupied by plankton in ml.

In displacement method, place the plankton sample in a measuring cylinder and make the volume to a desired level say 100 ml. Now pour the sample in a filter paper and collect the filtrate water in another measuring cylinder; note the volume of filtrate. The difference between the original volume and filtrate volume will be the exact plankton volume in ml.

PERIPHYTON

Periphyton are organisms, mostly algae, that are attached to the sediment surface (epipsammic and epipelic), stones (epilithic), woody branches or aquatic macrophytes (epiphytic). They are often abundant in the littoral zone where macrophytes dominate (see Wetzel 1983). Often benthic algae and other organisms get detached and occur also as plankton.

Several types of sanplers have been developed for samp;ing periphyton according to the nature of the substrate. Surber sampler, Hess sampler, or box sampler can be used in several cases. Any cylindrical coring device of a definite size for specific area of the book substrate can be used for soft sediments. Cobbles or pebbles can be taken out and examined separately. In case of macrophyes, plans have to be sampled and them periphytn examined on different parts. Algae are removed by scraping (by hard brush or sharp scalpel) from a small specified area of the substrate surface and the scarped material collected in a small vial or plate in a known volume of water. The sample is homogenised and examined under the microscope. Quantitative estimates can be made by weight method or counting method using a cell appropriate to the size of the periphytic algae. For details, refer to Wetzel and Likens (2000).

Periphyton play a key role in primary productivity, nutrient cycling, and food web interactions (Vadeboncoeur and Steinman 2002). They constitute an important food for fishes and other aquatic animals. Also, these algae play a major role in oxygenation of the water column, thereby degrading organic wastes and hence, influencing water quality. They also regulate nutrient cycles, particularly phosphorus (Dodda 2003, Azim et al. 2005).

ECOSYSTEM SERVICES OF PHYTOPLANKTON

i) Algae as primary producer

Phytoplankton play vital role in the freshwater aquatic ecosystems. Most of the algae are fundamentally autotrophic, act as primary producer, which convert water and carbon dioxide in the form of food and energy base for all aquatic organisms in the presence of sunlight and produce oxygen as by-product. The level of primary production by algae in fresh water aquatic system can be measured by fixed carbon per unit area with time (mgC/m^3h), which can vary from one environment to another. Availability of high nutrient load such as nitrogen and phosphorus in the eutrophic lakes will have high level of primary productivity at the surface. In contrast, low over all productivity in

mesotrophic and ologotrophic lakes, but productivity extends deeper into water column due to higher light penetration.

Blue green algae (Cyanobacteria) are very important primary producers, found in all type of freshwater environment, from high nutrient to low nutrient condition and phytoplanktonic/benthic habitats. These algae frequently dominate on the surface water. These algae in high nutrient condition can form nuisance bloom in mesotrophic to eutrophic environment. For example colonial algae *Microcystis*, *Anabaena* etc form massive growth in eutrophic condition (Bellinger and Sigee 2015) but lower number can also be found in the oligotrophic environment (Reynolds 1990). When nutrient levels become very high, a shift can occurs from colonial blue- green to green algae as major bloom forms. This type of situation can be seen in some of the fish culture pond due to high organic and manure application to enhance fish production. In this condition a short diatom bloom (*Navicula* and *Nitzschia*) is replaced during early summer by rapidly growing unicellular and small colonial green algae such as *Scenedesmus* and *Pediastrum.* Blue green algae have ability to adjust their buoyancy; they can float or sink depending on light conditions and nutrient supply. All plants, including all algae absorb Nitrate (NO_3^-) and /Ammonium (NH_4^+) from aquatic system to meet their demand from water. However, some of the algae (*Aphanizomenon*, *Anabaena*, *Nostoc* etc) absorb atmospheric nitrogen (N_2) and dissolve into the water and convert into ammonium through process called nitrogen fixation. Blue green algae are well adapted to phosphorus deficient situation because of their ability to absorb and store excess phosphorous.

ii) Algae as biological water purifier

Recently, algae have become significant organisms for biological purification of waste water due to their ability to accumulate plant nutrients, heavy metals, pesticides, organic and inorganic toxic substances and radioactive matters in their cells/bodies because their bioaccumulation abilities. Due to their symbiotic relationship exist among the bacteria and algae in the aquatic ecosystem; they release carbon dioxide and nutrients in aerobic condition by aerobic bacterial oxidation of organic matter. Algae utilize nitrogen and phosphorous for their growth may remove substantial amount of nutrient load from the aquatic environment. Therefore, algae act as significant component in the treatment of waste water. Increase dissolves oxygen concentration through photosynthesis and pH influence on phosphorous sedimentation, ammonium and hydrogen sulphur removal. Although wastewater is treated in pond via physical, chemical and biological processes and/or mechanical processes like aeration, there are also ponds completely based on processes of natural conditions. Removal efficiency of heavy metals by algae shows changes among species. In fact, many authors showed that by *Oscillatoria*, cadmium, copper and zinc by *Chlorellavulgaris*, lead by *Chlamydomonas* and molybdenum by *Scenedesmus chlorelloides* may remove successfully.

iii) Algae as bioindicators

Biological indicators (bioindicators) can be defined as presence of particular group of species or community in the environment at particular site, at particular point of time, provided information regarding physical and chemical parameters. Algae are diverse group of organisms, wide temporal and spatial distribution and their tolerance for particular environment. Algae is a vital component of aquatic ecosystem, because their nutrient requirement, rapid reproduction rate, short life span and quickly respond in the species community structure as well as their density due to slight change in the water chemistry of the environment. Hence algae can be an important component in the biological monitoring of water quality. Kolenti (1848) and Cohn (1853) was first to observed that freshwater algae composition have potential to change in the changing environmental conditions, because they found biota int he polluted waters were different from those in non-polluted water (quoted in Leibmann, 1962). Most of the Cyanobacteria grow at relatively higher temperature (early summer) and hight nutrient condition. Regular detection of an intense summer bloom of the colonial blue-green algae *Microsystis* and *Anabaena* is indicative of high organic nutrient (eutrophic) status of the aquatic environment. Phytoplankton abundance, growth and community composition, and photopigments as phytoplankton taxonomic groups and biomass has gained popularity recently in water quality monitoring and ecological assessment (Pinckey *el al.* 1954, Paerl *et al*., 2005).

iv) Algae and water pollution

Analysis of phytoplankton diversity and density of water sample collected from various aquatic ecosystems (Lakes, rivers, streams etc) provides information about the nutrient load and status of the water body. The algal species can provide potential information regarding general ecology and water quality, which can be useful in early warning sign of water deteriorating conditions. The nutrient load (nitrogen and phosphorus) from the point source/ non point source can be also analysed through the analysis of littoral algae. Wetlands are receiving inorganic compounds from various sources such as detergents, commercial fertilizer used in the agriculture and rain water runoff along with organic pollution from the domestic waste and other sewage related sources (Palmer, 1969). These organic and inorganic loads are in the aquatic system influence on the algal growth and resulting into bloom of nuisance and/ or toxin production.

v) Algae in trophic status of aquatic ecosystem

Algal abundance and density can be used as tool to determine the trophic status of the lake. Trophic status of the lake can be an indicator of the stage of lake in the process of natural ageing, termed as eutrophication. Productivity of the lake and species composition of phytoplanktonic algae in the epilimnion can be related to trophic status of lake.

- ❖ Oligotrophic: dominance of desmids (*Staurastrum, Closterium, Selenastrum*), Euglenophta (*Euglena*) and presence of some Dinophyta such as *Ceratium*, *Peridinium* is indicator of low nutrient and possibly high oxygen in the aquatic ecosystem.
- ❖ Mesotrophic: In the early and mid summer dominance of Blue green algae and green algae and Bacillariophtes (Diatoms)
- ❖ Eutrophic: In high nutrient load and possibly low oxygen situation during the mid-summer and possibly dominance of Blue green algae such as *Anabaena*, *Aphanizomenon* and *Microsystis.*
- ❖ Hypertrophic: This situation generally seen in the artificial culture pond due to high organic manuring, show throughout dominance of small unicellular algae with short life span.

IDENTIFICATION

Identification of all algae requires appropriate staining to distinguish between the major groups, and then microscopic examination under medium or high resolution, besides measurements of body size. Keys are available for only a few algal families. Illustrations of a few taxa are included here to show the range of variation, often within the genus (e.g., *Scenedesmus*)(Figures 5 and 6).

It is not our objective to provide keys to identification of numerous families and genera of different kinds of algae. More important publications for help in identification of the taxa are listed below:

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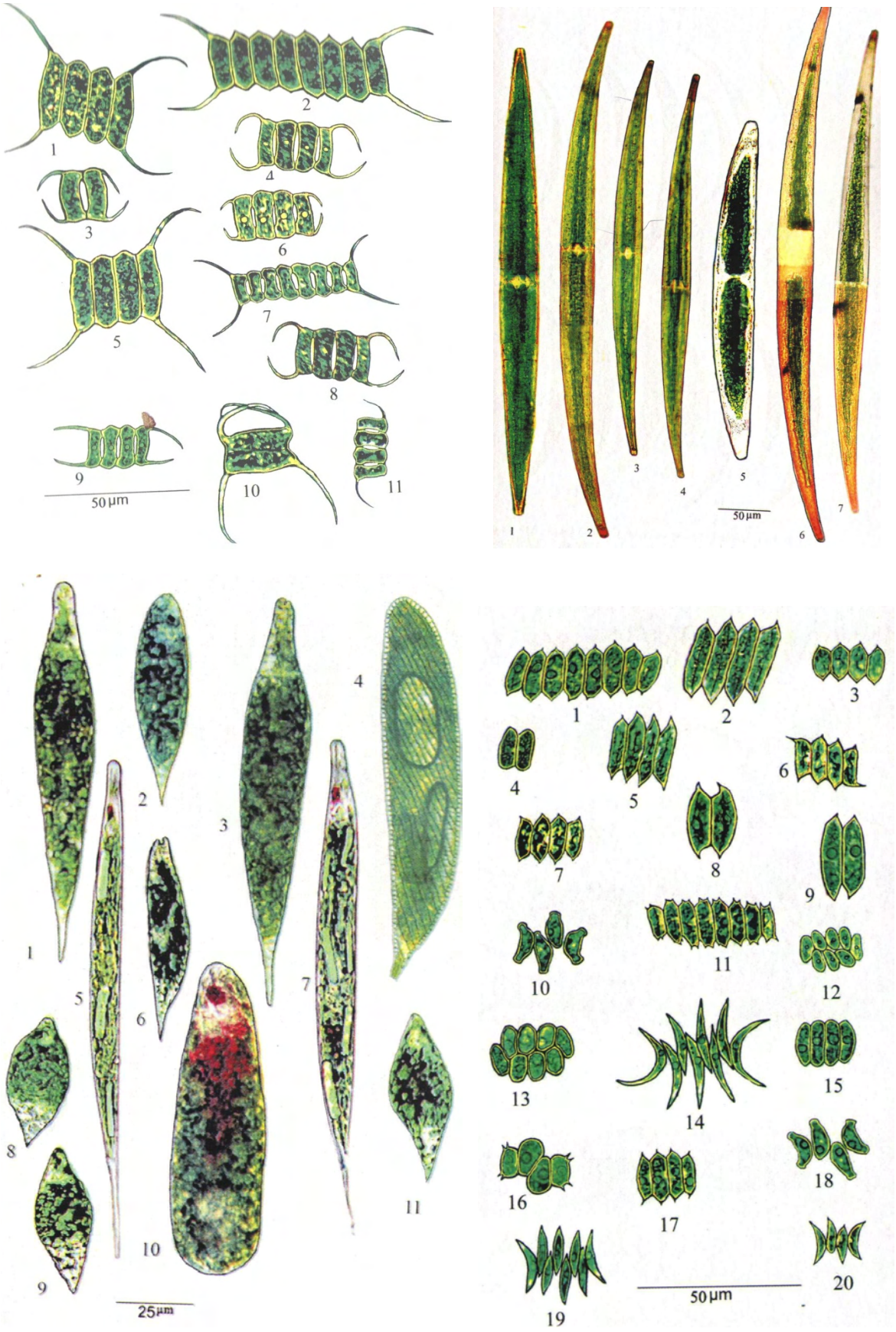

Figure 5: Variation in morphology of species within genera

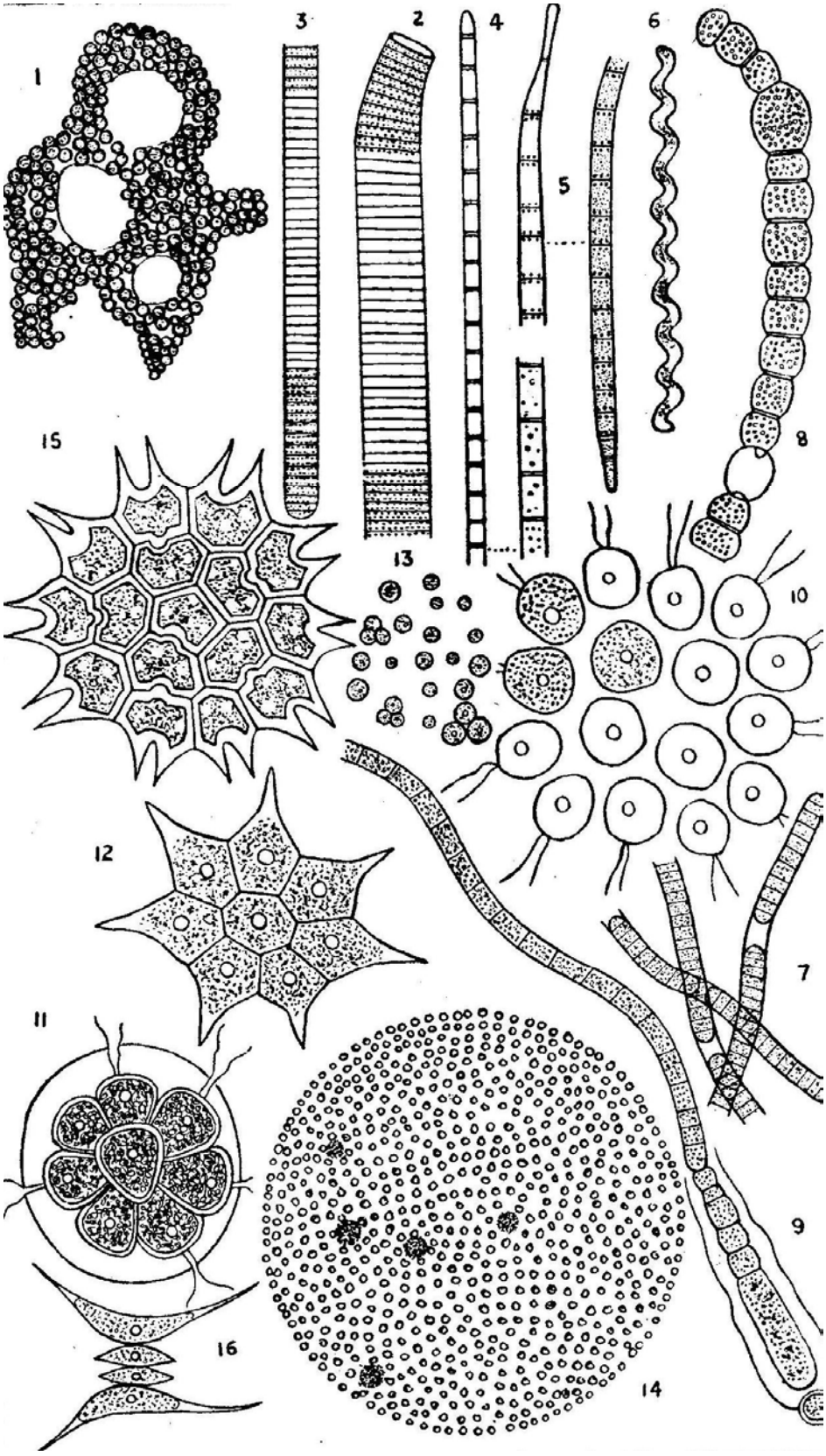

Figure 6. Different forms of algae: unicellular, colonial and filamentous

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RAPID ASSESSMENT OF BIODIVERSITY

ZOOPLANKTON

Suman Kumari

INTRODUCTION

Zooplankton (Greek: Zoon, animal; planktos, wandering) are diverse group of floating and drifting animals with limited power of locomotion. Majority of them are microscopic, unicellular or in multicellular forms with size ranging from a few microns to a millimeter or more. In addition to size variations, there are differences in morphological features and taxonomic position. Microscopic, Planktonic organisms with characteristics of animals are included under zooplankton division and includes Protozoa, Rotifera, Cladocera and copepoda. Among these zooplankton Rotifera, Cladocera and copepoda constitute nearly 90% of the total zooplankton population of freshwater ecosystem (Munshi *et al*., 2010) and play very important role as primary consumers in food chain of aquatic ecosystem converting energy from phytoplankton to a form that can be used by larger animals. Zooplanktons are integral components of aquatic food webs and contribute significantly to aquatic productivity in freshwater ecosystems. The success of zooplankton assessment and productivity would largely depend upon the use of correct methodology which involves collection of samples, concentration, fixation & preservation, identification, counting & computation of data and measurement of biomass.

QUANTITATIVE AND QUANTITATIVE ASSESSMENT

Following steps are involved in the quantitative assessment of zooplankton

Step-I: Collection of Sample
Step-II: Concentration of zooplankton
Step-III: Fixation and preservation
Step-IV: Identification
Step-V: Counting and computation of data of zooplankton
Step-VI: Assessment of biomass

STEP-I: COLLECTION OF SAMPLES

Collection of zooplankton sample involves following equipments depend on the aquatic system from which sample to be collected; Filtration of known volume of water through hand held plankton net, collection of known volume of water sample in bottles or, water samplers or by pumps. Method of sampling will largely depends on the objective of the zooplankton sample collection and the aquatic ecosystem from which sample will be collected. Success of sample collection mostly depends on the objective of zooplankton collection, selection of suitable equipments such as selection of suitable gear, mesh size of the gear, netting materials, time of collection, water depth of sampling site and sampling strategy (Vertical/ Horizontal hauling of net). For qualitative assessment of zooplankton need little more care because these animals are very sensitive towards even minor disturbance.

Bottles / water samplers: This method is used mainly for collecting smaller forms or micro-zooplankton.

Pumps: The gear is normally used on board the vessel/boat. The advantage of the method is that the volume of the water pumped is known.

Plankton nets: the most commonly used methods for zooplankton collection. This gear is suitable both for qualitative and quantitative studies. Collection of zooplankton can be made by horizontal, oblique and vertical hauls. The horizontal collections of zooplankton are mostly carried out for the surface and subsurface layers and in oblique hauls, the net is usually towed above the bottom while, vertical haul is made to sample the water column. The net is lowered to the desired depth and hauled slowly upwards

.

Water Bottle

Niskin bottles water sampler

Plankton net

Figure 1: Sample collection tools

STEP-II: CONCENTRATION OF ZOOPLANKTON

Concentration of sample can be done by three ways: Filtration: Plankton sample can be further concentrated by sieving it through a fine mesh or even through a membrane, Centrifugation: This method only suitable for nanoplanktonic collection. Zooplanktons being very small are very difficult to handle. For any type of study, their isolation, separation and transfer from one slide/reagent to another is required. These steps require special tools and skill. Animals can be isolated and transferred by specially designed microloops, droppers, needles, micropipettes etc.

STEP-III: FIXATION AND PRESERVATION

Once the sample is collected, it must be fixed and preserved as soon as possible. Various killing and preserving reagents have been used for cytological, anatomical and morphological studies. For taxonomic studies:

- Formalin: 2-10% (Prepared from 40% formaldehyde solution available commercially)
- 2-4% formalin: for Delicate form, Protozoa and Illoricate Rotifera
- 5-6% formalin: for medium size Cladocera and copepoda
- 8-10% formalin : for fairy shrimp

STEP-IV: IDENTIFICATION

For identification of zooplankton up to species level, dissection are mandatory and for these require a stereoscopic dissecting microscope, good quality glass slides, cover slips, stainless steel fine forceps, dissecting needles, pipettes and chemical reagents are required. Needle prepared from thin tungsten wires of 0.005 and 0.010 inches diameter are the best for the purpose. Correct species identification is prerequisite for understanding distributional pattern, seasonal variability and community structure of

zooplankton in an aquatic ecosystem. It is mandatory that Protozoa and Illoricate Rotifer be examined live while the other planktonic organisms may be taken from well preserve sample. It is a specialized work and requires patience, experience and sufficient published literature.

Taxonomic Characteristics of Protozoa

A group of animal, which comprises the single –celled microscopic animals, size range 5-500µm in diameter with more than 50,000 species have been described. They include amoebas, flagellates, ciliates, sporozoans, and many other forms. They are now usually treated as a number of phyla belonging to the kingdom *Protista.* Protozoan shapes vary from spherical to irregular shape and these shape found useful in species identification. They can be classified into three different group based on their shape:

- Ciliates: They have hair-like projections called cilia poking out around the edges of the protozoa. Most of the cilates swim along the water by beating their cilia in rhythmic pattern, like numerous tiny oars.
- Amoebae: Irregular projections of their cells called pseudopods (false feet), which can stretch out, bend and curve.
- Flagellates: whip-like projections called flagella poking out of their cells. They swim through the water by waving their flagella as fishes use its tail to push their body against water.

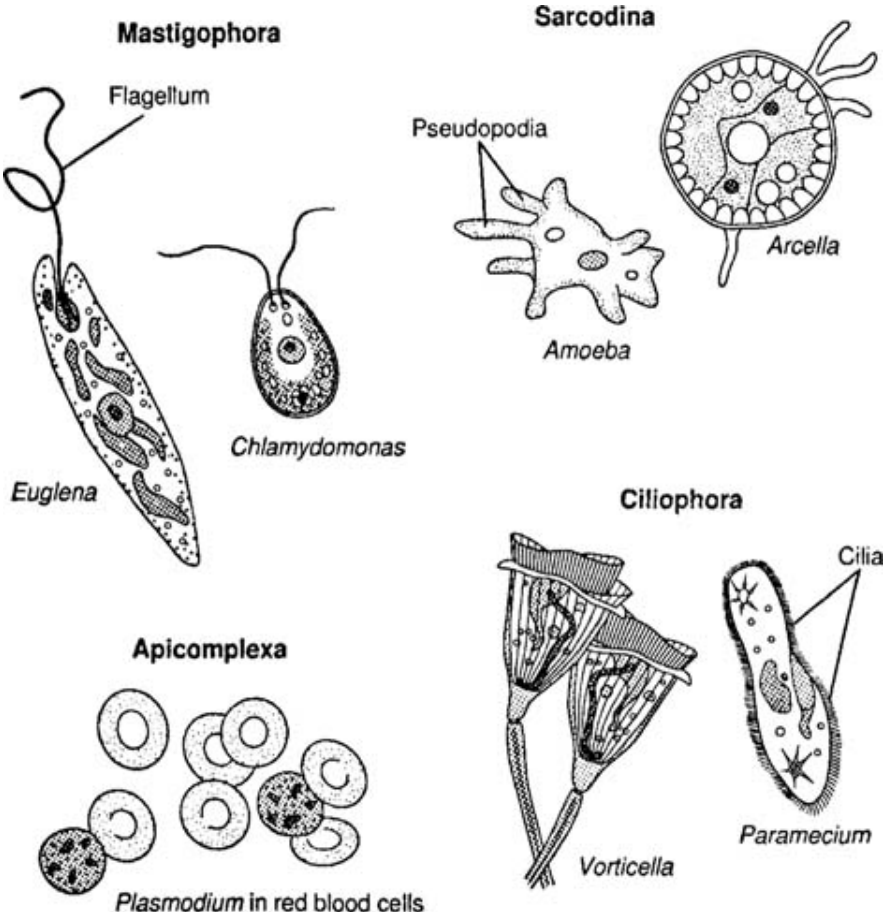


Figure 2. General morphology of protozoans (Source: www.cliffsnotes.com)

Taxonomic Characteristics of Rotifers

There are over 2000 species of rotifers in freshwater systems. They are so named for their distinct mouth, called a corona. It is used for both locomotion and filter feeding. Acording to Edmonson (1959) considered Rotifera is a phylum and divided into three classes; Seisonida, Bdelloidea and Monogononta of size range varies from 40-3500µm. Living Rotifers can be easily distinguished by their ciliated corona disc with synchronous beating of its cilia looks like a rotating wheel. Class Bdelloidea and Monogononta comprise higher number of species and dominant in the freshwater system. In the preserved specimens the mastax (trophy) is the only hard parts and characteristic feature of the rotifer, which not only confirm the rotifer but also useful in separating the class into orders and families or even up to species.

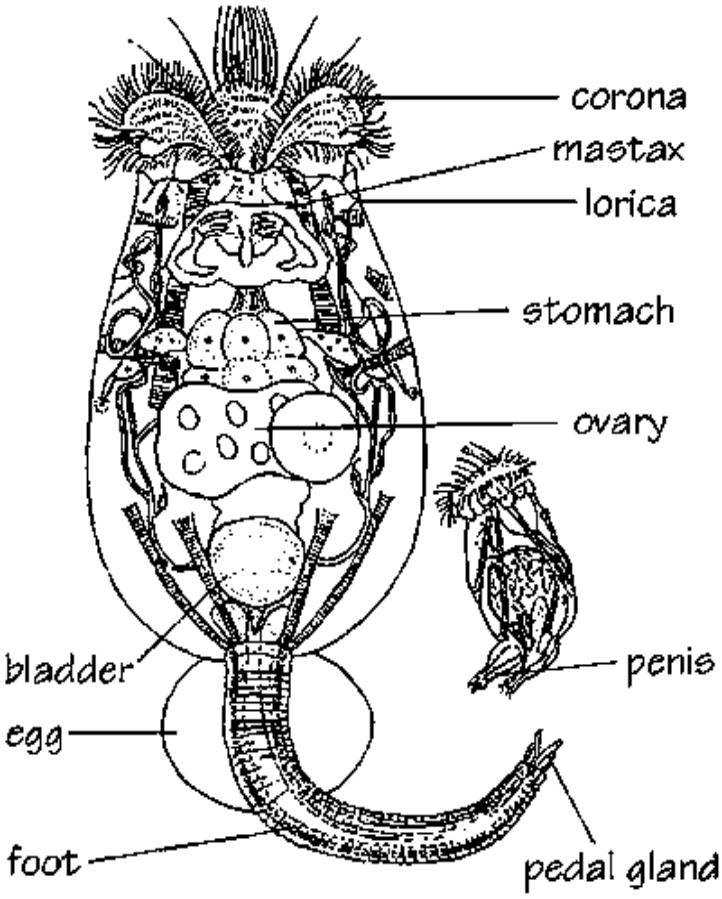


Figure 3. Morphology of *Brachionus*, modified from Koste, 1980

Taxonomic Characteristics of Cladocerans

The Cladocera is an order of Subphylum Crustacea and phylum Arthopoda and commonly known as water flea. Order Cladocera includes 620 species (marine and freshwater) so far. These small crustaceans are characterized by a two-valve carapace, or outer shell, covering most of their body. Size, shape and position with respect to the body , presence or absence of rostrum, its size and shape, presence or absence of cervical sinus, size of eye, shape and size of antennules, its attachment on head and position of setae (Figure 4).

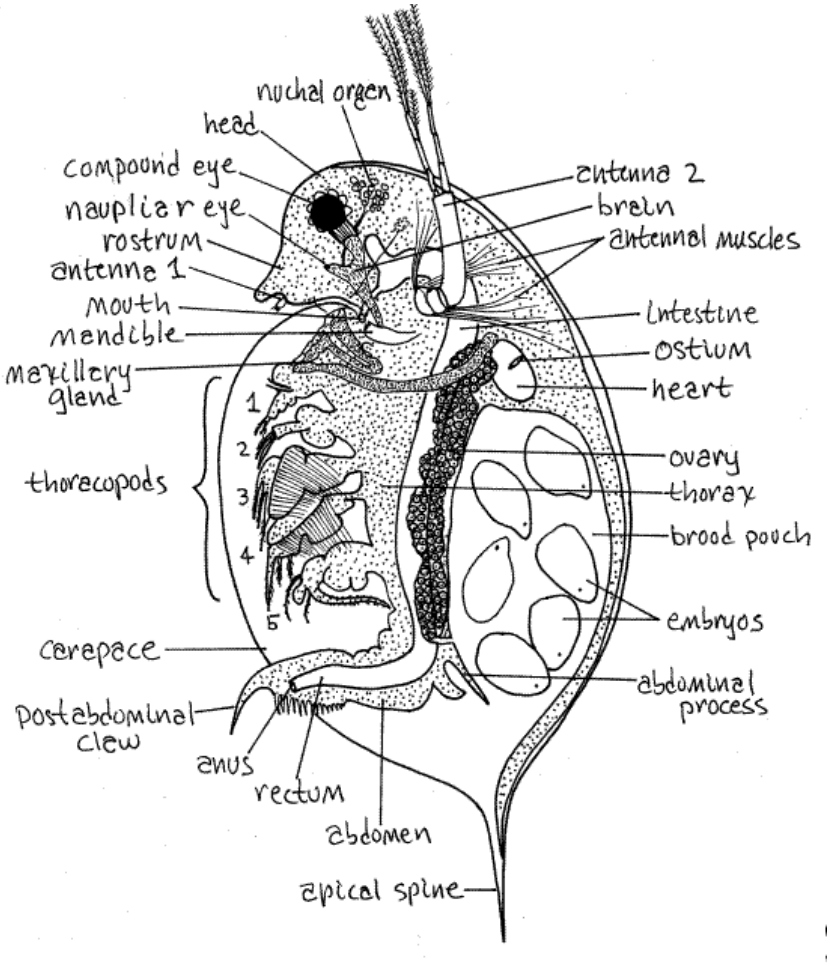


Figure 4. Female Daphnia Redrawn from Freeman & Bracegirdle (1971)
(Source: http://lanwebs.lander.edu/)

Taxonomic Characteristics of Copepods

Copepods are holopanktonic, stay planktonic for throughout their life. Most of the copepods possess a single median compound eye, usually red in colour at the centre of the transparent head and have two pairs of antennae, the first pairs conspicuous and usually long.

STEP-V: COUNTING AND COMPUTATION OF DATA

The common taxa observed there are Protozoans, Rotifers, Cladocerans, Copepods (adults and life history stages), decapods larvae, mysids etc. The counting should be done under the microscope with the help of Sedgwick Rafter cells and when the specimen of a particular group is seen, a tally mark is made on the sheet. All the specimens present in the subsample are counted with proper records on the data sheet and computation of data is done simultaneously. The total number of specimens is later calculated for the whole sample depending on the percentage of subsamples examined. Image analysis systems are being tried for rapid counting of common taxa and their species.

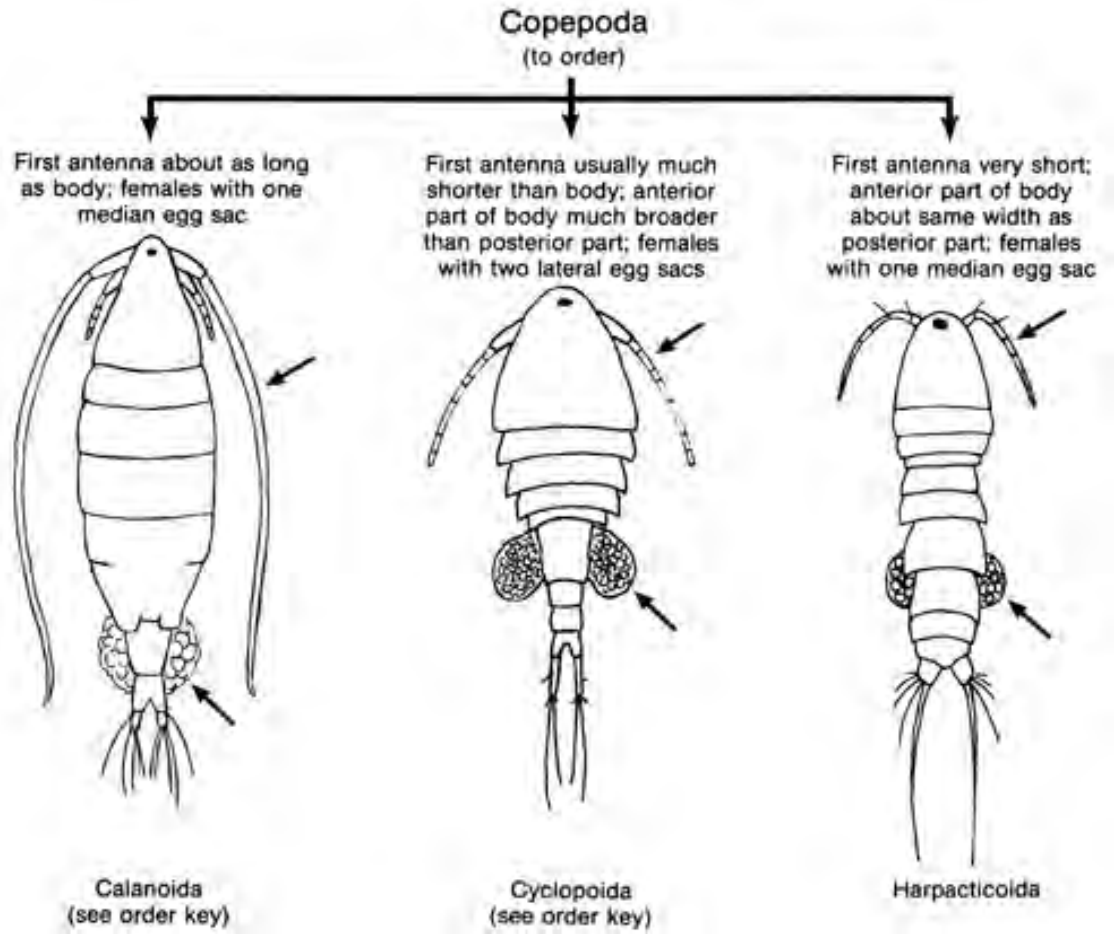


Figure 3. Drawing made by Jesse Claggett (Source: http://fusedjaw.com/)

STEP-VI: ASSESSMENT OF BIOMASS

The term biomass denotes the live weight or the amount of living matter present in the zooplankton sample. The value obtained is used to evaluate the secondary productivity and fishery potentials of the study area. The biomass is estimated by the following methods:

1. Volumetric (displacement volume and settling volume) method
2. Gravimetric (wet weight, dry weight and ash free dry weight) method
3. Chemical method

Volumetric (Displacement Volume and Settling Volume) Method

The total zooplankton volume is determined by the displacement volume method. The volume measurements are easy to make in the field or laboratory. In this method the zooplankton sample is filtered through a piece of clean, dried netting material. The mesh size of netting material should be the same or smaller than the mesh size of the net used for collecting the samples. Filtered zooplankton with the help of spatula transferred to known volume of 4% formalin solution. The displacement volume is obtained by recording the volume of fixative in the measuring jar displaced by the zooplankton. The plankton is allowed to settle for at least 24 hours before recording the settled volume.

Gravimetric Method

The weight measurement should be done in laboratory, carried out by filtering the zooplankton. The interstitial water is usually removed by blotting paper. While blotting, due care should be taken not to exert too much pressure as to damage the delicate organisms or specimens. The zooplankton weight (gm) is taken on predetermined or weighed filter paper or aluminium foil. The dry weight method is dependable as the values indicate the organic content of the plankton. Analysis such as the dry weight is determined by drying an aliquot of the zooplankton sample in an electric oven at a constant temperature of 60ºC. The dried aliquot is kept in desiccators until weighing. The values are expressed in milligram per litre. Ash free dry weight method is also occasionally used for biomass estimation.

Chemical Method:

In this method, the live zooplankton samples are dry frozen. Before analysis, the samples are rinsed with distilled water. Measurement of constituent elements such as carbon, nitrogen, phosphorus and biochemical elements viz. protein, lipid and carbohydrates are made. Sometimes the biochemical values of a particular taxon and species are undertaken to evaluate food energy transfer at higher trophic levels. The calorific content of the plankton can be used as an index of zooplankton biomass.

Biomass (standing stock)

After estimation of zooplankton biomass the standing stock values are converted into per cubic meter is calculated as follows:

1. Volume of zooplankton (ml/m^3):

$$\frac{\text{Total volume of zooplankton}}{\text{Volume of water filtered (V)}}$$

2. Wet weight of zooplankton (g/m^3):

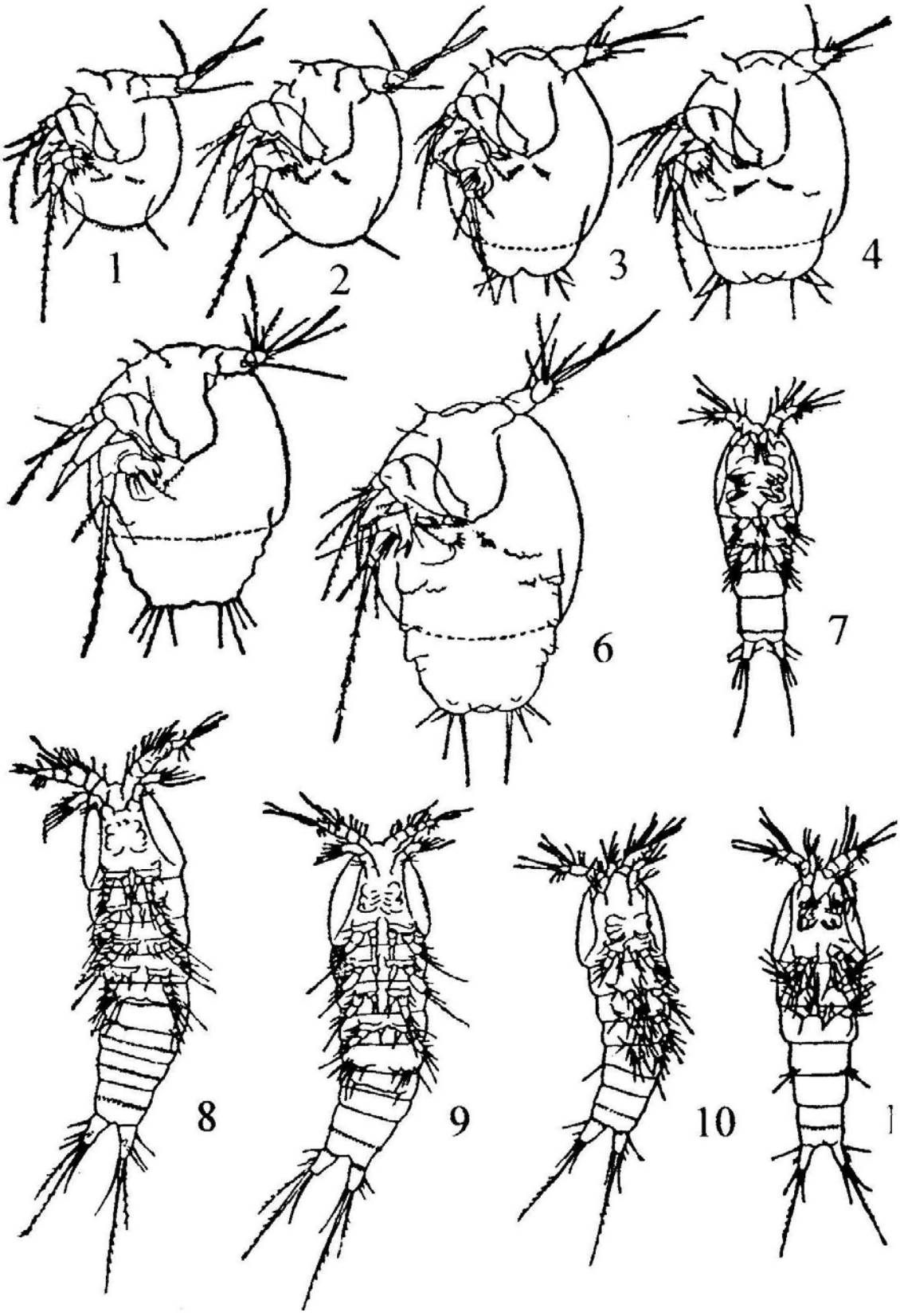
$$\frac{\text{Total wet weight of zooplankton}}{\text{Volume of water filtered (V)}}$$

3. Dry weight of zooplankton (mg/ m^3):

$$\frac{\text{Total dry weight of zooplankton}}{\text{Volume of water filtered (V)}}$$

ECOSYSTEM SERVICES

Through their consumption and processing of phytoplankton and other food sources, zooplankton play a role in aquatic food webs, as a resource for consumers on higher trophic levels (including fish). Being typically small, zooplankton can respond rapidly to increases in phytoplankton abundance such as during the spring bloom. Zooplanktons are integral components of aquatic food webs and contribute significantly to aquatic productivity in freshwater ecosystems. Zooplankton can also act as a disease reservoir. Crustacean zooplankton have been found to house the bacterium Vibrio cholerae, which causes cholera, by allowing the cholera vibrios to attach to their chitinous exoskeletons. This symbiotic relationship enhances the bacterium's ability to survive in an aquatic environment, as the exoskeleton provides the bacterium with carbon and nitrogen.



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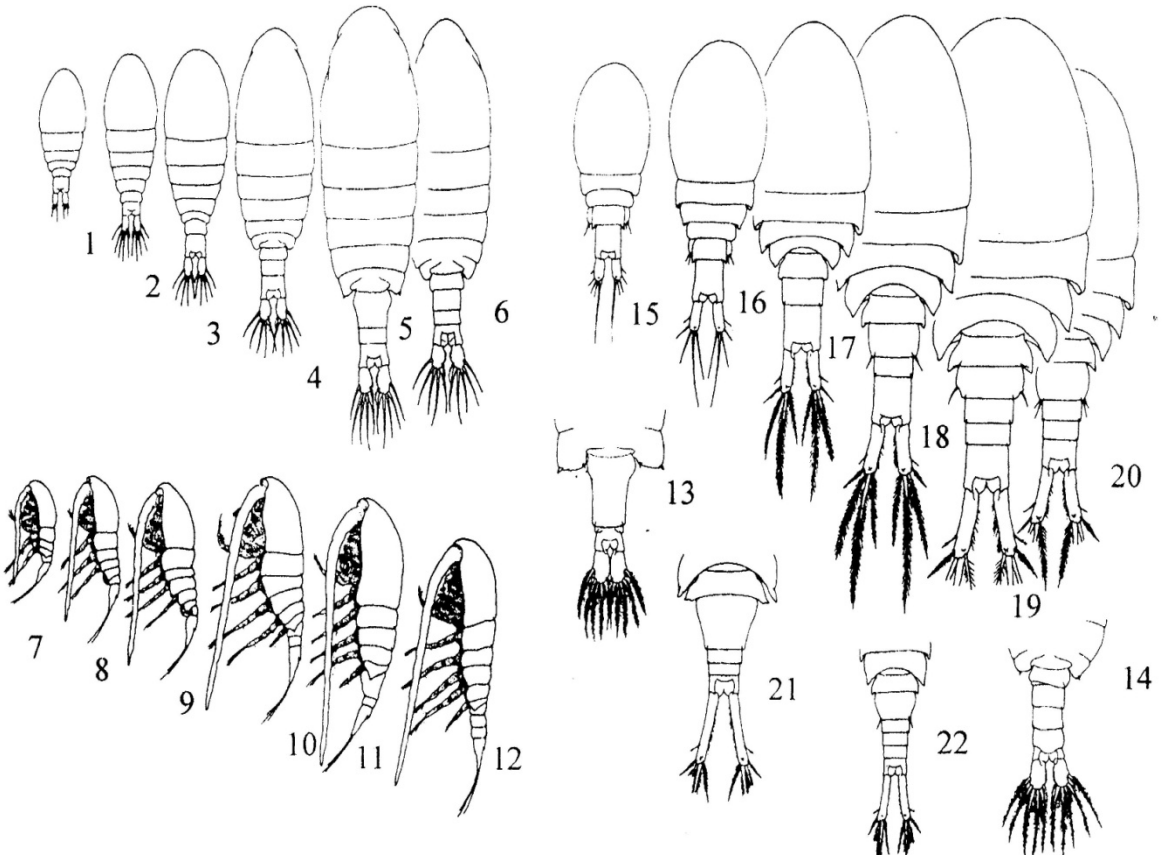

Figure .

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RAPID ASSESSMENT OF BIODIVERSITY

BENTHIC MACROINVERTEBRATES

Deep Narayan Shah, Subodh Sharma and Ram Devi Tachamo Shah

1. BENTHIC MACROINVERTEBRATES

Benthic macroinvertebrates are animals which have no backbone and can be seen without the aid of a microscope. These animals occur in almost all aquatic environments. They live on and in the waterlogged soils; and on, under and around rocks and sediments at the bottom of lakes, rivers, and streams. Therefore, macroinvertebrates are often regarded as "benthos" (community of bottom dwelling organisms).These animals are the most diverse of all aquatic organisms. They are generally the immature or adult stages of many insects (such as mayflies, stoneflies, caddisflies, dragonflies, damselflies, midges, and beetles), arachnids, crustaceans, molluscs, leeches and annelids. Among the approximately 125,000 freshwater species described globally, more than half are benthic macroinvertebrates including insects, some 10,000 crustaceans and 5000 mollusc species (Balian et al. 2008, Strayer and Dudgeon 2010).

Crustaceans and non-insects live entire life in the water. Aquatic insects have complex life cycles and live in the water only during certain stages of their development. They go through one of two kinds of metamorphoses i.e., *incomplete metamorphosis* (Fig. 1a) or *complete metamorphosis* (Fig. 1b). Incomplete metamorphosis has three main stages: egg, nymph and adult while complete metamorphosis has four stages: egg, larva, pupa and adult.

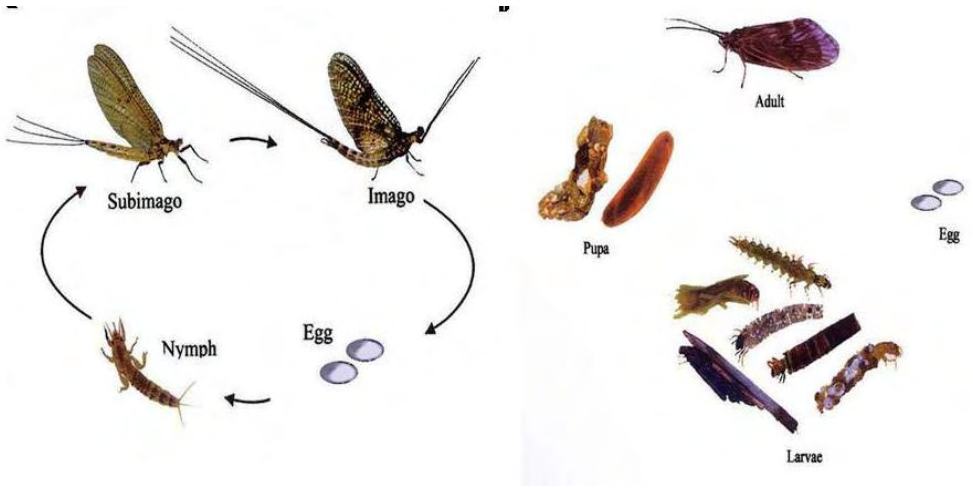

Figure 1. Life cycle of (a) Mayfly (Incomplete metamorphosis) and (b) Caddisfly (Complete metamorphosis). Image © *2014 John Constantine's (chalk streams) Bucket.*

Macroinvertebrates play an important role in the food webs of wetlands and other aquatic ecosystems. They constitute significant part of food of larger animals such as fish, amphibians and water birds. They are also involved in the breakdown of organic matter and in nutrient cycling.

Macroinvertebrates can be classified based on their feeding adaptations and/or food preferences into functional feeding groups (Table 1). Each group has specific adaptations for obtaining and eating food. For instance, plants are eaten by herbivorous mayfly who is eaten by a predatory insect (Megaloptera). A fish, in turn, eats the Megalopteran larvae and an osprey eats the fish.

Table 1. Functional Feeding Groups of benthic macroinvertebrates

Feeding Strategy	Food Category
I. Shredders	Dead leaves/live macrophytes
II. Collectors	Fine organic particles (live/dead)
Filter feeders	Particles in water column
Miners	Buried particles
Browsers	Bottom surface deposits
III. Scrapers	Live benthic algae (diatoms)
IV. Piercers	Live filamentous algae
V. Predators	Other invertebrates + small fish

2 SAMPLE COLLECTION

Sampling of macroinvertebrates requires pre-planning in order to standardize the sampling effort i.e., maintain consistency in the effort expended for each sample. In a sampling team there should be at least one expert who can identify specimens of a taxonomic group to the possible level (family/genus), is familiar with current sampling and collection methods. One should have a good understanding of local geography, ecology and community issues.

The sampling of wetland macroinvertebrates is preferable during pre-monsoon season (March-May). For special studies (i.e., to identify seasonal variation, impact assessment etc.) may require sampling at other periods.

2.1 Sampling Equipment

The following equipment are essentially required for sampling the macroinvertebrates:

- Hand/pond net (D-frame net; mesh size 500 µm), the frame attaches to a long handle (Fig. 2).
- Peterson grab sampler (Fig. 3)
- Waders or gumboots, depending on the depth of water
- Utility/work gloves
- Measuring tape
- White tray
- Bucket (max. 10 litre capacity)
- Air tight plastic sample containers (usually 500 – 1000 ml capacity)
- Vials, forceps, small plastic Petri dishes
- Preservative (Ethanol or formaldehyde)
- Sample container labels
- Pen and pencil (waterproof)
- Field notebook
- Protocols
- GPS unit and spare batteries
- Power glass
- Camera and spare batteries

2.2 Sampling

In the wetlands (lentic ecosystem), benthic macroinvertebrates are associated with littoral, sublittoral, and profundal habitats of which the littoral habitat is usually more diverse. In the littoral habitat, the vegetation and substrate heterogeneity provide an abundance of microhabitats occupied by a varied fauna. The littoral habitat is highly variable due to seasonal influences, land use patterns, riparian variation, and direct climatic effects. The sublittoral habitat lies below the area of dense macrophyte

beds and above the typical thermoclines. This habitat lacks the heterogeneity of the littoral habitat and is also less subject to influences. The profundal habitat is more homogeneous and is usually dominated by three main groups of benthic organisms: the chironomid larvae, oligochaete worms, and phantom midge larvae.

Benthic macroinvertebrates have contagious (i.e. clumped or patchy) distribution making quantitative sampling difficult as it requires large numbers of samples to achieve reasonable precision in estimating population abundance (Rosenberg and Resh 1993).

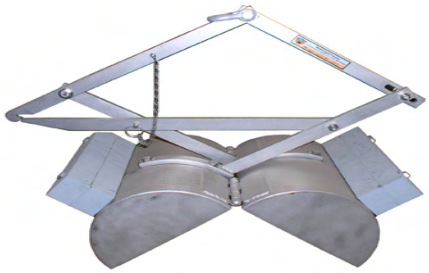

Figure 2 (Left): D-frame net, mesh size 500 µm. The net is ideal for scraping wetland bottoms (in mud or gravel beds), collecting bottom dwelling organisms, or sweeping in thick vegetation.
(source: http://www.coleparmer.com/).

Figure 3 (Right): Petersen Grab (Sample area: 305 x 305 mm, volume 9890 ml). The sampler is very versatile for all types of hard bottoms such as sand, gravel, marl and clay. (source: http://www.wildco.com/.)

Procedure

1. Fill up the site protocol (Annex I) for documenting major habitats and other associating factors. Take digital photos of the site and record the photo number on the site protocol.
2. For locating sample sites, measure the perimeter of a selected water body (pond/lakes/ wetlands) from bathymetric maps. Divide the perimeter evenly into approximately 20 survey sites. Take one sample from each selected site.
   OR, divide the 20 sampling efforts among the 5 habitat types. The number of samples from each habitat type may be determined by the relative proportion of the habitat types based on visual inspection.
3. In the littoral zone, a kick and sweep collection method is preferable. A standard D-frame kick net with a mesh of 500µm size is used.
4. Ensure that the sampling net and bucket/sieve are clean.
5. Approach the selected area slowly in order to minimize accidental disturbance.
6. Start the sampling at a depth of 1-metre and slowly walk towards the shore. Bump the net against the bottom substrate to dislodge and collect the organisms from the sediment. The sampling can be standardized to time (e.g., 3 minutes at each site).

- For multi-habitat sampling, sample all inundated microhabitats at each site using D-frame net by jabbing the net into the wetland substrate and quickly sweeping upward. Make sure to collect aquatic macroinvertebrates from areas having emergent vegetation, aquatic macrophyte beds consisting of floating and/or submerged plants, and areas between vegetation hummocks.
- Other techniques used for sampling macroinvertebrates in wetlands include benthic samplers (e.g., Petersen grab, Petersen 1918), and passive samplers such as artificial substrates (Batzer et al. 2001).

7. Transfer the sampled material to a white tray or bucket approximately half full of water. Wash or pick all animals off the net.
8. Rinse and remove any unwanted large debris items (e.g., stones, sticks, leaves) that may not fit into the sample container or will absorb and diminish the effectiveness of the preservative.
9. Transfer the sample to the sample container.
10. Add preservative (70% ethanol).

11. Place a label on the side of the sample container. The label should include the site code/name, date, sample type, etc using a permanent marker. If a single sample is divided between two or more sample containers, mention additional information on the label to indicate the container number, for example Jar 1 of 2.

2.3 Sample Preservation

Samples should be preserved for later sorting and identification in the laboratory. The fluid most commonly used for preservation of aquatic macroinvertebrates is ethyl alcohol (ethanol). Also, the samples are preserved in 4% formaldehyde, or in combination with the alcohol, although these invariably are unpleasant or hazardous substances. However, addition of 1–4% formalin to ethanol improves the effectiveness of ethanol as a preservative. Formalin (formaldehyde) is a fixative that helps to maintain the colour and shape of macroinvertebrates. It is recommended to use the ethanol-based preservatives (70–90% aqueous solution) because they are comparatively safe and specimens preserved in ethanol can further be used for genetic analysis. If samples are to be kept for more than a week or two before processing, the preservative should be replaced to maintain the preservative concentration. This is most important for organic-rich samples.

2.4 Sample Processing

In the laboratory, samples may be divided into subsamples for sorting and identification of organisms. Full counts of the samples provide the most accurate estimate of the abundances of individual taxa. Sub-sampling protocols involve systematic sorting, identification and counting of a pre-defined portion or number of animals in a sample. In sub-sampling, a scan for rare taxa is required to complete the species list. When the samples are either excessively large or have large numbers of organisms, they may be sub-sampled on the basis of either volumetric method (Wrona et al. 1982) or weight-based method (Sebastien et al. 1988) or the spatial sample-splitting method (Marchant 1989) to save processing time.

Procedure

1. All samples collected from the field should be recorded in a “laboratory log”.
2. Preserved samples must at least be stored for two weeks before being treated further. The preservative fluid needs to be replaced to maintain the preservative concentration. This is particularly important for organic-rich samples.
3. Clean the sieves and stack them in the bottom of a sink, with the larger mesh size sieve on top and fine mesh size sieve on the bottom.
4. Tip the sample into the top sieve using water to wash all material from the sample container. Ensure that the preservative is decanted from the samples thoroughly with tap water. This step is to remove the preservative and split the sample into several fractions by size of debris and animals to make it easier to find macroinvertebrates amongst the debris.
5. Do not use too much water pressure from the pipe as animals could be damaged. Rinse the animals attached to large organic material.
6. Invert the contents of each sieve into a separate white tray and wash all material off the sieve into the tray. Avoid too much material in each tray. If necessary, use additional trays for the finer fractions so that animals can be seen clearly amongst the debris.
7. The material is spread thinly over the bottom of a white tray and covered with a thin layer of water for picking out the animals. Work systematically across each tray starting from coarse to fine size sieve material. Pick up the animals gently using soft forceps and place them separately into different Petri dishes according to taxonomic groups. Do not include aerial adult insects, terrestrial invertebrates, empty mollusk shells, insect pupae, caddisfly cases, or exuviae.
8. Store the animals into a vial containing 70% ethanol for storage and quality control (QC). Place a label in the vial noting the site code/name, date, sample type, and collector’s name.
9. On completion of sample processing, the sample residue can be preserved in its original plastic container with original label.

10. For identification, place the organisms of each taxon encountered into separate Petri dish to identify the specimen to the lowest possible taxonomic resolution by examining under a dissection microscopes (5x or 10x magnification) or binocular microscopes (100x magnification). After identification place the animals into vials containing 70% alcohol for storage and Quality Control. Preserved samples must be stored in dark and at low temperatures to minimise the loss of colour.

Identification Keys

The following identification keys can be used for identifying the organisms:

Dudgeon, D. (1999) Tropical Asian Streams: Zoobenthos, Ecology and Conservation, 1st edition. Hong Kong University Press, Hong Kong.

Nesemann, H., Sharma, S., Sharma, G., Khanal, S., Pradhan, B., Shah, D.N. & Tachamo, R.D. (2007) Aquatic Invertebrates of the Ganga River System, H. Nesemann.

Nesemann, H., Tachamo Shah, R. D. & Shah, D. N. (2011) Key to the larval stages of common Odonata of Hindu Kush Himalaya, with short notes on habitats and ecology. Journal of Threatened Taxa 3: 2045-2060.

Yule, C.M. & Sen, Y.H. [eds] (2004) Freshwater Invertebrates of the Malaysian Region. Academy of Sciences Malaysia, Kula Lumpur, Malaysia.

2.5 Quality Control

To ensure data quality, inspections are needed to control errors (that normally occur in sorting and taxonomy). Ten percent of samples may be selected at random for re-examination by another taxonomist. Errors can also occur when entering data on to the computer; hence, particular attention should be paid to data entry.

2.6 Safety

In field:

1. One should be cautious around bank mud, boulders, bedrock or large woody debris to avoid injury.
2. For sampling during high water level, best professional judgment should be used to obtain samples.
3. Waders and specialized wading boots should be utilized when conducting biological sampling to remain dry and get protection against contaminants and natural irritants (i.e., biting insects, poison ivy, snakes).

In laboratory:

1. Process the samples preserved with formalin under controlled conditions i.e, under fume extractor or in a good aerated room.

3 BIOLOGICAL ASSESSMENT

Benthic macroinvertebrates are among the most commonly used organisms for assessing and monitoring the condition of running and stagnant water systems worldwide (Rosenberg &Resh 1993, Morse et al. 2007, Sharma and Rawat 2009, Li et al. 2010, Shah et al. 2011). The primary goal of biomonitoring is to determine the status of the water resource, evaluate the causes of degradation, assess the relative impacts of degradation on biotic assemblages and determine the effectiveness of control and mitigation programs (Barbour et al. 1999, Morse et al. 2007). Benthic macroinvertebrates live continuously in the water and exhibit varying responses to changes in water chemistry, physical habitat and water level fluctuation. Different types of macroinvertebrates tolerate different levels of pollution. In general, some organisms are pollution-sensitive (cannot tolerate organic pollution and are associated with clean, well-oxygenated water), some are pollution-tolerant (survive and commonly thrive in heavily polluted water) and some are intermediate or facultative (capable of living under a wide variety of conditions). The response of each macroinvertebrate to environmental stressors

produces measurable and often predictable shifts in abundance and composition at the community level. Biological responses are observed to assess the impact of external factors on ecosystems and their development over a period, or to ascertain differences between one location and another (Markert et al. 1999).

Advantages of benthic macroinvertebrates in bioassessment

- They occur in high abundances making them relatively easy to sample,
- They have relatively larger body size making them easier to identify,
- They are taxonomically and ecologically highly diverse,
- They live from few months to years allowing them to integrate short- and long term pollution exposures,
- They have limited mobility than fishes preventing them to escape from occasional pollutions,
- Many taxa are highly sensitive to changes in water quality, water level fluctuations and habitat changes, and
- Their community occupies the large portion of aquatic food web and forms a vital link between aquatic plants, algae, and leaf litter to the fish species and even birds.

Biological assessment is based on the comparison with empirically defined reference conditions which are best established through systematic monitoring of actual sites that represent the natural range of variation in "minimally" disturbed water chemistry, habitat, and biological conditions (Gibson et al. 1996).

A variety of indices have been developed to represent the ecological status of the ecosystems. The commonly used ones are described below.

Nepal Lake Biotic Index (NLBI, Shah et al. 2011):

It was developed for evaluating the stagnant water body's status in Nepal. NLBI is a biotic score index system that contains numerical scores (also called 'tolerance scores') to specific "indicator" organisms at a particular taxonomic (family, genus and species) level. The taxa tolerance scores range between 1 and 10 representing highly pollution tolerant and highly pollution sensitive taxa (see Shah et al. 2011). Once the NLBI index is calculated, the wetland ecological condition (e.g., high, good, fair, poor, and bad) can be described by referring to the transformation scales (Annex II).The index is calculated as following:

$$\text{NLBI} = \sum_{i=1}^{n} \left(\frac{TTSi}{n} \right)$$

where, TTSi is the taxa tolerance score of taxon i and n is the total number of scored taxa.

Multimetric Approaches

Multimetric indices are set of variables/metrics that include structural and functional components of an ecosystem (e.g., species composition, feeding types, substrate preferences, current preferences, life cycle parameters, pollution tolerance) (Li et al. 2010, Shah et al. 2011). The purpose of multimetric approach is to aggregate the information about the elements and processes of aquatic communities. For a metric to be useful, it must be (1) ecologically relevant to the biological assemblage or community under study and to the specified program objectives; and (2) sensitive to stressors and provide a response that can be discriminated from natural variation.

Diversity indices:
Diversity indices consider three components of community structure namely, species richness (number of species present), evenness (uniformity of distribution of species in a site), and abundance (total number of individual) to assess the impact of stressors on the biological community. The commonly used diversity indices are:

Shannon-Wiener diversity index (H)
This index is a quantitative measure that reflects the diversity,and simultaneously takes into account how evenly the individuals are distributed among the species found (Shannon and Wiener 1949). The usefulness of this diversity index for assessing water quality is based on the assumption that clean waterbodies have high diversity index, and in contrast, polluted waterbodies are interpreted to have low diversity index. According to Wilhm (1970), "H" usually varies between 3 and 4 in clean-water waterbodies and is usually less than 1 in polluted-waterbodies. This index places relatively little weight on rare species and more weight on common species (Krebs 1994). Its value ranges from 0, indicating a low level of diversity, to a maximum of 1-1/s.

$$H = -\sum_{i=1}^{s} (p_i)(lnp_i)$$

where "pi" is the proportion of individuals in the "i^{th}" taxon of the community and "s" is the total number of taxa in the community.

Simpson's Index of Diversity (1-D)
Simpson's index (D) is calculated as (after Pinder et al. 1987):

$$D = \frac{\sum n(n-1)}{N(N-1)}$$

where "n" is the total number of organisms of a particular species in a site and "N" is the total number of organisms of all species in a site.

The value of Simpson's index of diversity ranges between 0 and 1. The greater the value indicates higher the sample diversity.

Species Richness
Species Richness equals the total number of taxa represented within the sample. The healthier the community is, the greater the number of species found within that community. Species Richness increases with increasing habitat diversity, suitability, and water quality (Plafkin et al. 1989).

Margalef's index:
This index is used as a simple measure of species richness (Margalef 1958; Wilhm and Dorris 1968).

$$Margalef's\ Index = \frac{(S-1)}{\ln N}$$

S = total number of species, N = total number of individuals in the sample, In = natural logarithm

Pielou's Evenness Index (e):
This index calculates the evenness of species i.e., how close in numbers each species in an environment is (Pielou 1966).

$$e = \frac{H}{\ln S}$$

H = Shannon – Wiener diversity index, S = total number of species in the sample, In = natural logarithm

4 ECOSYSTEM SERVICES

Macroinvertebrates are a major contributor to several ecosystem services of wetlands (Table 2). They play a major role in maintaining ecosystem functions such as energy flow and nutrient cycles. Macroinvertebrates process live plants and plant litter inputs by shredding them into particles for other consumers. Covich et al. (1999) show that the benthic fauna mediate biogeochemical transformations, thereby prevent the buildup of carbon in the sediments and consequently the deoxygenation of bottom waters. They also sequester and move contaminants and excess nutrients from groundwaters and sediments while influencing the flux of greenhouse gases.

Many macroinvertebrates are of great economic importance; for example, molluscs (e.g., *Bellamiya bengalensis*), shrimps (Palaemonidae), and crabs are consumed as a source of protein in Asia including India and Nepal (Fig. 4). Adults of larger water beetles such as Dytiscidae (e.g., *Cybister*, *Eretes*) and Hydrophilidae (e.g., *Hydrophilus*); Odonata nymphs (e.g., Aeshnidae); and water bugs (e.g., Belostomatidae) are also consumed in different parts of China and Thailand. Larger macroinvertebrates are good source of food for fishes, otters, water fowls, birds, turtles, and frogs.

Many water beetles and bugs prey on mosquito larvae, thereby helping in controlling the mosquito population. Macroinvertebrates also control rapid spread of algal blooms. Worms and mussels purify water by filtering water. For example, one mussel can filter 20-70 liters of water per day (Xerces Society 2011). Some high altitude Oligochaetes (e.g., *Tubifex tubifex*) can transmit parasites and cause lethal disease to trout (Brinkhurst 1997). Some macroinvertebrates have high educational value. For instance, *Pila globosa* are used as a teaching and learning tool in biology.

Figure 4. Macroinvertebrates as food; Odonata nymph (a), Crab (b), Prawn (c), Shrimps (d), and Molluscs (e).

Table 2. Ecosystems services provided by benthic macroinvertebrates

Types of services	**Services**
Supporting services	Role in nutrient cycling Predator-prey relationships
Regulatory services	Processing of organic matters, Pollination
Cultural services	Existence values, Educational, Recreation
Provisioning services	Food (e.g., nymphs of Odonates, Water beetles, Crabs and Snails), Genetic resources

5. BENTHIC MACROINVERTEBRATES GROUPS

5.1 Key to freshwater macroinvertebrate groups

The key is adopted from Dudgeon (1999), Yule and Sen (2004) and Nesemann et al. (2011).

1. Macroscopic (visible to the unaided eyes) organism, body is soft and unsegmented with a ventral muscular foot and covered with unsegmented calcareous shell ….……..snails, clams, mussels: **Phylum Mollusca. .2**
   – Macroscopic (visible to the unaided eyes) organism, body soft and without shell…….…..…… **.4**
2. Body covered by single shell (may be reduced)…… **Class Gastropoda…..3**
   – Body covered by a bi-valved shell …… **Class Bivalvia**
3. Shell aperature can be closed by a horny operculum attached to the foot; shell shape usually turbinate or turriculate with dextral coil; relatively large (usually >15 mm long) …… **.Order Prosobranchia**

   – Operculum lacking; shell is rather thin and delicate and may be globose; relatively small (≤ 5 mm long) ……..……. **Order Pulmonata**

4. Body unsegmented, thin and flattened (worm like)……..…… ……....…….**5**
   – Body slender and long (may be worm like).……..……....…….... **.8**
5. Body dorsoventrally flattened or rounded in cross-section, soft, compressible……...…….**6**
   – Body typically rounded in cross-section, not soft, very slender……. **9**
6. Body thin and long, usually rather rounded in cross-section, extremely contractile and extensile. With an eversible proboscis which is proturbed from anterior end; three pairs of eyes; Sometimes brightly coloured; rare ….. **Phylum Nemertea** (Ribbon worms)
   – Body thin and flattened, pressed to the substrate. Move with a gliding motion. Often with a pair of anterior eyespots..... **Phylum Platyhelminthes** (Flatworms)…**7**
7. Larger worms (>5 mm long)……**Suborder Tricladida**
   – Tiny worms (Usually 1-5 mm long)…….... **Microturbellaria**
8. Tiny, slender worms, usually <10mm long, typically white, body tapering at both end and lacking external segmentation; Move with a whip-like wriggling ……..…….....……... **.Phylum Nematoda** (Round worms)
   – Body long (>20 cm), extremely thin and thread like bodies, anterior and posterior ends of body blunt (not tapering). Usually dark brown to black in colour; rare …..……..horsehair worms: **Phylum Nematomorpha** (Horsehair worms)
9. Body segmented; without jointed limbs……...……..…….….**10**
   – Body segmented, with jointed limbs……...… **Phylum Arthropoda** ….**11**
10. Body soft and worm like with more than 15 visible segments; suckers may have at both anterior and posterior sections of body; if suckers are absent then segments bear

paired, fleshly, lateral outgrowths or fine bristles (chaetae).....true worms and leeches: **Phylum Annelida** (segmented worms)

– Body with fewer than 15 visible segments, often hardened and divided into two or three discrete regions (e.g., head, thorax and abdomen). Sclerotized mouthparts visible (may be reduced and not protruding from thorax); Prolegs (unjointed stubby limbs) may be present on some segments…….. **Phylum Arthropoda: Insecta** – larvae of Diptera and Coleoptera

11. Three pairs of legs…….. **Class Insecta**

– More than three pairs of legs……. **12**

12. Four pairs of jointed legs……..…… **Class Arachnida**….**13**

– More than four pairs of jointed legs or other appendages; two pairs of antennae..... **Subphylum Crustacea**…**14**

13. Body globose, tiny (<4 mm). Head, thorax and abdomen fused, antennae lacking, ……..…. **Subclass Acari** (water mites)

– Body larger (up to 30 mm). Body divided into cephalothorax (fused head and thorax) and abdomen. antennae lacking. Legs long ……**Subclass Araneae** (Spiders)

14. Abdominal appendages present. Carapace present, extending down over sides of thorax and enclosing branchial chamber. Five pairs of legs. Eyes on stalks. Quite large (> 40 mm body length) …..... **Order Decapoda** (Shrimps, carbs)…..**15**

– Without carapace. Eyes not on stalks. Five or seven pairs of legs. Relatively small (< 40 mm body length) ……..……..……...……..**16**

15. Abdomen folded beneath the cephalothorax. Body rather flattened and rounded. Rostrum reduced or lacking. Tail fan absent …..… **Suborder Brachyura** (Crabs)

– Abdomen extended and well developed. Tail fan present. Conspicuous rostrum projecting in front of eyes…….....… .**Suborder Caridea** (Shrimps)

16. Body dorsoventrally flattened. Seven pairs of legs with the posterior ones longer than anterior ones…….……..**Order Isopoda**

– Body laterally compressed. Five pairs of legs …… **Order Amphipoda**

Keys to the orders of aquatic insects

1. Small insects usually 1-2 mm, with forked spring-like apparatus under abdomen……..…..….**Collembolla**

– Larger insects, never with a forked spring-like apparatus under the abdomen……..……**2**

2. Flap-like wing buds present on the thorax……..……. **3**

– External flap-like wing buds absent……..……**8**

3. Mouthparts in the form of a segmented beak….....……...**Heteroptera**

– Mouthparts not in the form of a segmented beak…… …… **4**

4. Mouth covered by an elbowed mask-like labium……..……**Odonata**

– Mouth not covered by a mask-like labium……...… **5**

5. Middle and hind legs terminate in a single tarsal claw. Abdomen ends with three (mostly) long filiform ‘tails’. Single or double gills attached to the dorsal side of abdomen …….……..…….... **Ephemeroptera**

– Middle and hind legs with two tarsal claws; abdomen without dorsal gills … 6

6. Hind legs elongated with expanded femora and modified for jumping; shore insects…… O**rthoptera**

– Hind legs not elongate and modified for jumping……. .**7**

7. Body oval and flattened; head much smaller than prothorax…….. .**Blattodea**

– Body somewhat slender; head not so small, thoracic gills in the form of tracheal tufts (present in most cases)……..…….. **Plecoptera**

8. Three pairs of true legs present on the thorax…… **9**

– Without three pairs of true legs on the thorax; “false legs” present on the thorax or abdomen……..……. **14**

9. Underside of abdomen with false which bear hooklets arranged in circles or ovals…… . **Lepidoptera**

– Abdomen without circles of hooklets on underside……..……. **10**

10. Mouthparts in form of needle-like styles……...….**Neuroptera**

– Mouthparts not in form of needle-like styles……..… **11**

11. Abdomen ends in a pair of short or long, fleshly prolegs (sometimes fused together) that end in a single hook……. **Trichoptera**

– Abdomen ends variously, but never in a pair of fleshy prolegs each ending in a single hook……..… .**12**

12. Abdomen hardened and lacks well-developed lateral filaments … ……..… .larvae **Coleoptera** (in part)

– Abdomen is fleshly with well-developed lateral filaments…… **13**

13. Abdomen ends in a pair of prolegs, each with a pair of hooks (family: Corydalidae); or ends with a single slender terminal filament (family: Sialidae)……..……..**Megalopetra**

– Abdomen ends variously but never in a pair of prolegs each having a pair of hooks, although there may be two pairs of prolegs which each have hooks in some of the family Gyrinidae……..……..... .**Coleoptera** (in part)

14. Headless, legless, living as parasites, often inside the eggs or bodies of other insects…… .**Hymenoptera**

– Not headless parasites…….. **15**

15. Without a distinct, sclerotized head capsule…… **Diptera** (in part)

– Larvae with a distinct, sclerotized head capsule……...…… **16**

16. Body oval or elongate, no complex structures at posterior end ……..……. **Coleoptera** (family Curculionidae)

– Body shape complex, with a breathing tube or other structures at the end of the abdomen ……..…… **Diptera** (in part)

5.2 DESCRIPTION OF GROUPS

5.2.1 Ephemeroptera (Mayflies)

Habit and Habitat: The nymphs live primarily in streams under rocks, decaying plants, or in the sediment. Few species live in shallow parts of lakes.The nymphs can grow to be 3 to 4 mm in length. Most species feed on algae or diatoms, but a few species are predatory. Mayflies have a very short but interesting life cycle consisting of three stages- egg, nymph, and adult. The lifespan of an adult mayfly is very short and varies depending on the species. The primary function of the adult is reproduction. The adult mayflies are usually found near vegetation and are attracted to lights.

Distinguishing Characteristics - Mayflies have fringed gills on each segment of the abdomen. Their mouth parts are classified as chewing and they only have one claw. They usually have two or three tails that are of the filamentous shape.

Ecological Role - Mayflies have an important specific ecological role in the ecosystems of many freshwater bodies. These nymphs graze and consume large amounts of algae and other build ups of organic particles in the water. This helps the water maintain its nutrients cycle and not become too polluted with fast spreading organisms like algae. Mayflies are very abundant when the water is fresh, oxygenated, and clean. Trout and salmon depend the most on mayflies for food. Some other significant predators are birds and dragonfly nymphs. When the mayflies die, they lay themselves in the water. This is easy and free food for the predators to get, supporting many organisms. Ultimately, this role benefits the food chain. The larvae are "ecological indicators" of good water quality too.

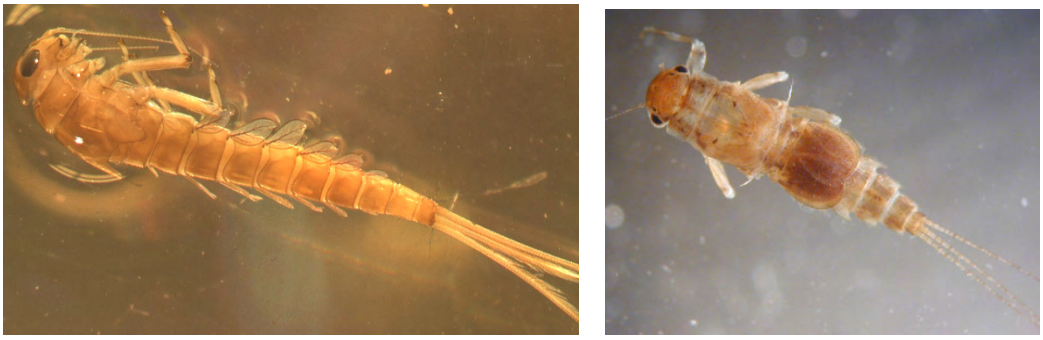

Figure 5. Ephemeroptera; Baetidae (a) (Image © RDT Shah) and Caenidae (b).

Key to families

1. Gills on the abdominal segment II -large and plate like (operculate), touching along the dorsal midline and covering all of the succeeding (III-VI) gills. Hindwing pads lacking ……. Caenidae
2. Body is cylindrical. Abdominal segment II- VII possessing plate-like gills, terminal filament is much reduced than the cerci (later filaments). Antennae are longer (twice as long as the width of the head) .....……..…… Baetidae

5.2.2 Trichoptera (Caddisflies)

Habits and Habitats: The larvae of Trichoptera are aquatic and live in the bottom of the streams and littoral zones of lakes and reservoirs. They inhabit on surfaces of large rocks, interstitial spaces among gravels, and sands, debris, twigs, woods, mosses and roots of macrophytes. Most species have thread like abdominal gills. They may be herbivores, scavengers or predators. The herbivores and scavengers build protective cases from their own silk and stones, twigs or leaf fragments. Predatory species are mainly free-living or spin silken structures in water.

Distinguishing Characteristics: Trichoptera larvae are resembled to Lepidoptera (moths). They have long antennae. Body shape ranges from eruciform (caterpillar-like) to campodeiform (tapered at the ends). Abdomen usually enclosed in protective cases. Abdominal segments consist of thread-like gills.

Ecological Role: Many Trichoptera larvae are sensitive to pollution. Fish and other aquatic vertebrates feed on Trichopteran larvae. A few species have been recorded as pests in rice paddies.

a

b

Figure 6.Trichoptera: Lepidostomatidae (Image © 2011 Robert G Henricks), Leptoceridae (Image © 2008 Mark Melton).

Key to families

Antennae very long and prominent, at least 6 times as long as wide; Ventral apotome divided into two pieces of approximately equal size (one anterior, the other posterior), and a mid-ventral suture in the central part of the head capsule connects these two parts……Leptoceridae

Tarsal claws of the hind leg differs from those on the anterior limbs, which are modified to form short setose or slender filament; flattened case of (mainly) sand grains with lateral flanges and a dorsal cowl …… ……..…… Molannidae

Antennae situated close to the anterior margin of the eye, dorsal hump on the abdominal segment I lacking, prosternal horn present; abdominal segment VIII with a fleshy lateral lobe on the either side; case often 4 sided and constructed fromplant detritus…… Lepidostomatidae

Meso- and metanotalsclerites closely appressed on midline or fused medially; claws of all thoracic legs of similar size; each anal claw, curved to about 90 degrees and with row of tiny teeth on inner margin; larva in fixed retreat. Widespread in shores of lakes ……. .Ecnomidae

5.2.3 Diptera (True Flies)

Habits And Habitats: The Order Diptera is divided into two suborders: Nematocera and Brachycera. Most Nematocera larvae are aquatic. They are highly diverse in stream ecosystems. The Nematoceran family Chironomidae is considered as the most diverse and abundant group of benthic macroinvertebrates. Many Brachycera larvae are terrestrial and few are aquatic. Aquatic Brachyceran larvae are commonly found in lakes, ponds, swamps, and phytotelemata. Feeding habits are highly variables and differ between species of single family. Some are herbivores or predators but most feed on dead organic matter or parasitize other animals, especially vertebrates, molluscs, and other arthropods.

Distinguishing Characteristics: All dipteran larvae do not possess legs. Body may be culiciform or vermiform. Head capsule is present with reduced mouthparts, only present as mouth hooks.

Ecological Roles: The adult Diptera have a greater economic impact on man kinds than any other insect groups. Some species are pests of agricultural products, others transmit diseases to humans and domestic animals. They are also beneficial in pollination of flowering plants, decomposition of organic matter, or as bio-control agents of insect pests. Diptera larvae are also good source of food for many large aquatic invertebrates.

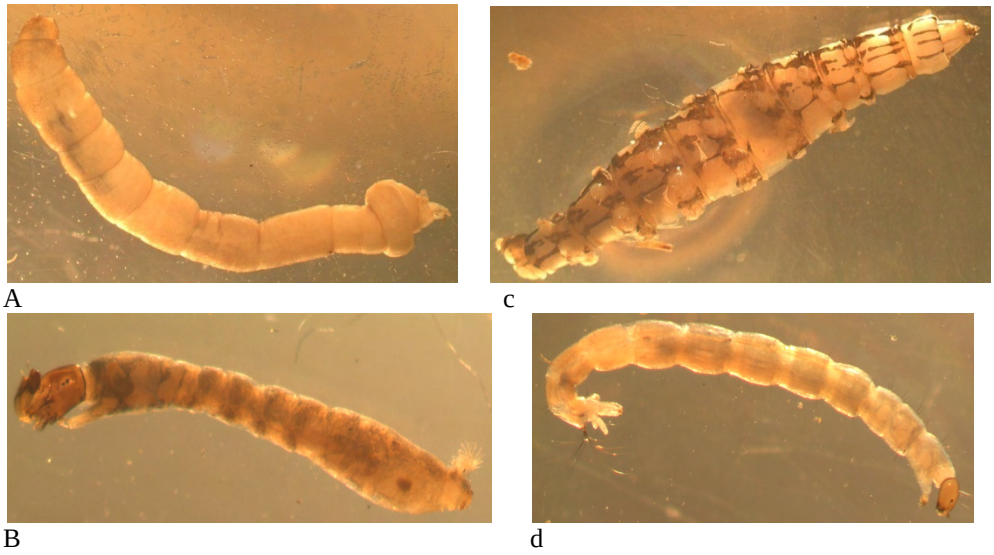

Figure 7.Diptera: Limoniidae (a), Simuliidae (b), Tabanidae (c), Chironomidae (d). Image © RDT Shah.

Key to families

1. Body dorsoventrally flattened; head capsule visible; integument (skin) leathery; body tapered at anterior and posterior end; Long setae present at the posterior end ……..…….. Stratiomyidae
– Body cylindrical; head capsule may or may not be visible; integument not leathery; long setae may or may not present at posterior end…… 2
2. Head capsule completely visible and separated from thorax…… 3
– Head capsule absent or retracted to thorax…… .8
3. Prolegs present either only on prothorax or at prothorax and at posterior end of abdomen…… 4
– Prolegs absent…….. 5
4. Prolegs present only on prothorax; posterior third of abdomen is broadest; posterior end of abdomen with a circle of hooks……. Simuliidae
– Prolegs present on both prothorax and posterior end of abdomen, posterior third of abdomen is not broadest; body is narrow and elongated; posterior end not terminating with a circle of hooks but hooks may be present on posterior prolegs…… ……. Chironomidae
5. Thoracic segments fused and formed broadest……..… 6
– Thoracic segments not as above; do not form broadest…….…..…. 7
6. Antennae long and used as a prehensile organ; two prominent air sacs in each of thoracic segment…… Chaoboridae
– Antennae shot and not as above; prominent mouth brushes present on either side of labrum……..… .Culicidae
7. Body is tapered at anterior and posterior ends; body segment with 2-3 secondary divisions; body colour is white or grey or brown……..... Psychodidae
– Body is very thin; slightly tapered at both ends; body segment without secondary divisions; slender, white and worm like ……..……. Ceratopogonidae
8. Much of head capsule visible; mandible moves against each other on a horizontal plane…….....…….. Tipulidae
– Head capsule lacking or completely reduced; mandibles moves parallel to each other on a vertical plane ……....…….. 9

9. Posterior end of abdomen terminating with an unpaired extendable respiratory tube…..…….. 10
– Posterior end of abdomen without an unpaired extendable respiratory tube…… 11
10. Anterior portion of body blunt; respiratory tube as long or even longer than the larvae and not bifurcated at the end; anterior spiracles, if present, borne on short to long tubular stalk ……..…… Syrphidae
– Anterior portion of body tapered; respiratory tube as long or slightly shorter than the larvae and bifurcated at the end …….....… Ephydridae (in part)
11. Body wrinkled; most of segments with rings of tubercles; posterior end of abdomen terminating with spiracles that is surrounded by lobes……..... .Scomyzidae
– Body is not wrinkled; body with distinct prolegs in most of abdominal segment 12
– Body is not wrinkled; body without distinct prolegs……. 14
12. Head slightly visible with antennae and palpi; seven or eight pairs of prolegs present, the posterior/most pair being longer than the anterior ones; abdominal end terminates with 1-4 lobes bearing short seate……..... Empididae
– Head is not visible; abdominal end terminates with varying shape…… 13
13. Abdominal end terminates with a pair of short respiratory tube…..… .Musidae
– Abdominal end terminates with an unpaired respiratory tube that is bifurcated at the tip; posterior prolegs may or may not be present……. Ephydridae (n part)
14. Body tapered at both ends; terminal process absent; abdominal segments ringed by a girdle of at least six pseudopods around each segment…..……. Tabanidae
– Anterior portion of body tapered; posterior abdomen terminates with 4 lobes ……..... Dolichopodidae

5.2.4 Coleoptera (Water Beetles)

Habits and Habitats: The vast majority of order coleoptera or beetles are terrestrials. Only about 10000 species out of 350000 known coleopteran species are related to aquatic environment in one or more of their development stages. In aquatic environments, beetles habitat range from temporary pools or mud flats to mountain streams. Beetles are generally herbivores, scavengers or predators, however, some beetles in adult stages do not feed at all. The feeding behaviors may be same or vary between larvae and adult stages. For instance some beetles are predatory in their larval stages while become herbivores at their adult stages.

Distinguishing Characteristics: Body of larvae may be campodeiform (slender), scarabeiform (grub-like, c-shaped body) or elateriform (wireworm, cylindrical). Head is well-developed with ocelli and chewing mouthpart. Three pairs of thoracic legs are present. Abdominal prolegs are absent.

Ecological Role: Water beetles (e.g., Elmidae, Scirtidae) are used as indicators of water quality, water types and endangered habitats. Water beetles control water plants such as water hyacinth. Dytiscidae are predatory in nature and play significant roles in controlling mosquitoes.

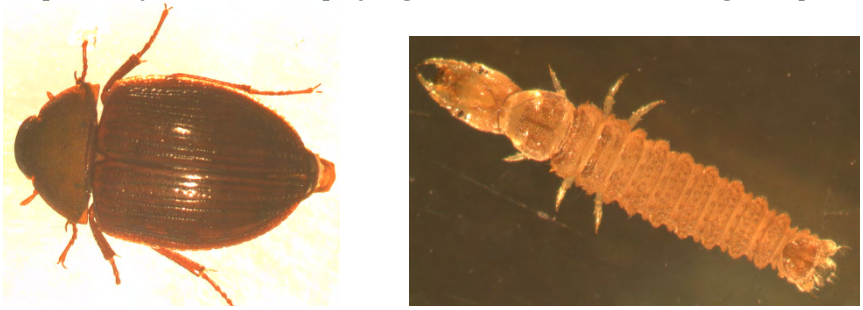

Figure 8. Coleoptera: Gyrinidae (a), Hydrophilidae (b). Image © RDT Shah.

Keys to adult beetles

1. Head extended in front of eyes to form a distinct snout of variable length (always definitely longer than wide); antenna inserted on snout, geniculate between scape and pedicel…….…….….Curculionidae
- Head anteriorly not extended into a distinct snout; antennae not geniculate between scape and pedicel……...……..2
2. Third tarsal segment bilobed……..Chrysomelidae
- Third tarsal segment never bilobed…….……...3
3. Middle and hind-legs strongly modified (short and flat,oar-like); much shorter than fore-legs. Head with two pairs of well-developed eye; one on dorsal side and one on ventral side. Antenna with setose; enlarged pedicel……..… .Gyrinidae
- All legs approximately equally long; head usually with only one pair of eyes……..4
4. Underside with conspicuously elevated metacoxal process; usually drop-shaped, apically acuminate…… Noteridae
- Metacoxal process-if present-never conspicuously elevated…….. 5
5. Metasternum with characteristics lateral wings. Metacoxae large; posteriorly with a paired posterior metacoxsal process…… .Dytiscidae
- Metasternum never with characteristic lateral wings; Metacoxae smaller…….. 6
6. Antenna not seen from above and dorsally covered by lateral extension of head; pubescent antennal club with 3 segments; abdomen usually with 4-5 distinctly visible sternites…….…. .Hydrophilidae
- Antennae of varying appearance; antennal base exposed; seen from above; claws always longer than penultimate tarsal segment…….…. .7
7. Antenna very short; with 5-13 segment; pedicel greatly enlarged; fronto-clypeal suture absent…… Dryopidae
- Antenna long or short, 7-11 segments, second segment never greatly enlarged. Fronto-clypeal suture usually present……...……. Elmidae

5.2.5 Hemiptera (True Bugs)

Suborder Heteroptera

Habits and Habitats: Most Heteroptera species (true bugs) are aquatic at their larval and adult stages. They are grouped into Gerromorpha (semi aquatic bugs) and Nepomorpha (aquatic bugs) according to their habitat preferences. Most Gerromorpha species live on surface of water while Nepomorpha species live beneath water surface. The habitats include ponds, lakes, streams, rivers and pytotelmata (small water pools made by living plants). They are predator and feed on other small aquatic invertebrates.

Distinguishing Characteristics: The Heteropteraare characterized by presence of typical forewings and scent glands. Their forewings are hardened at the base and membranous at the tips. Abdominal and metathoracic scent glands are present in Nymphs and adults, respectively. Mouthparts are modified into beak-like rostrum which is used for piercing and sucking.

Ecological Role: Some Heteropteraare used as indicator species in ecological assessment of water bodies. Heteroptera control the population of mosquitoes and other dipterans by feeding them. Some families of Heteroptera such as Corixidae are important food sources for fishes in fishery industries. Dried Corixidae are used as food for aquarium fishes (Yang et al. 2004). Some species of Heteroptera are consumed as food by local people in Thailand, Combodia and China (Yang et al. 2004).

A b c d e

Figure 9. Heteroptera: Helotrephidae (a), Micronectidae (b), Belostomatidae (c), Gerridae(d), Corixidae (e).Image © H Nesemann.

Key to families

1. Antennae exposed and longer than head; Hind-coxae small, cylindrical or conical; coxal cavity socket-like……. Gerromorpha…… 2
- Antenna shorter than head, inserted beneath eyes, with at most only the tips visible from above……..…… .Nepomorpha ……. 6
2. Head distinctly prolonged, eyes situated halfway along the head Hydrometridae
- Head not distinctly prolonged; eyes situated at base of head ……. 3
3. In long-winged forms, scutellum exposed; forming subtriangular, rounded or transverse plate behind pronotal lobe, apterous or short or short-winged forms with abdominal scent gland on tergum IV ….…… 4
- In long-winged forms, scutellum not visible, hidden by pronotal lobe; apterous or short-winged forms lacking abdominal scent gland…… 5
4. Antenna 4-segmented; bucculae well developed; reaching base of head; tarsi 2-segmented ……..…… Hebridae
- Antenna clearly 4-segmented; bucculae absent or poorly developed; tarsi 3-segmented ……..……..…… Mesoveliidae
5. Head with median longitudinal groove on dorsal surface; male fore-tibiae usually with a comb of short spines along inner margin; middle femora scarcely or not extending beyond tip of abdomen; hind-femora usually stouter than middle femora …… …. Veliidae
- Head without median longitudinal groove on dorsal surface; male fore-tibiae usually with a comb; middle femora usually extending well beyond tip of abdomen; hind-femora usually more slender than middle femora …… …… Gerridae
6. Apex of abdomen with paired respiratory processes ……...… 7
- Apex of abdomen without paired respiratory processes ……. 8
7. Respiratory processes cylindrical. Rigid and no-retractable, usually long and filiform; body either cylindrical or ovoid and flat …… ……... Nepidae
- Respiratory processes cylindrical, strap-like and retractable; body never cylindrical, always ovoid and flat…….…… …….. Belostomatidae
8. Body and fore-wings with transverse dark lines. Rostrum broadly triangular, non-segmented transversely striate, appearing as apex of head. Fore-tarsi with a single segment, spoon-or scoop-like, fringed with long stiff setae ventrally Corixidae
- Body colouration never as above. Rostrum cylindrical, short or long, obviously segmented, not transversely striate. Fore-tarsi segmented or not, not scoop-like or fringed with long stiffe setae … ……..… 9
9. Fore-legs not raptorial. Dorsum usually strongly convex or inversely boat-shaped……...…... 10

- Fore-legs raptorial. Dorsum usually flat; head and prothorax never fused; head usually longer than wide and produced in from of eyes. Antenna short. Rostrum cylindrical, short and thick, not surpassing prosternum ……..... …. Naucoridae

10. Body elongated, wedge-shaped, usually over 4 mm long; hind-legs elongate, oar-shaped, with two reduced and inconspicuous claws……. . Notonectidae
- Body oval, robust, less than 3.5 mm long; hind-legs not oar-shaped, usually with two distinct claws…….…… 11

11. Antenna 3-jointed; head and pronotum separate, cephalonotal sulcus straight, complete, and distinct……..… Pleidae
- Antenna 1- or 2- jointed; head and pronotum fused, cepahlonotal sulcus not straight, incomplete, often indistinct…… Helotrephidae

5.2.6 Odonata (Dragonflies and Damselflies)

Habits And Habitats: Odonates spend about 70–95 % of their life span in water. They inhabit both in lentic and lotic environments. Odonata comprises two suborders: Zygoptera and Anisoptera. They can be distinguished easily from the anal appendages. Nymphs of Odonates generally live underwater of ponds, lakes and flowing streams. They may be burrowers, clingers or live beneath detritus, aquatic vegetation, sand or mud. Odonates possess a unique labial palp that covers the mouth parts and use for catching pray. All odonates are predators at larval and adult stages.

Distinguishing Characteristics:
Anisoptera: Body is robust and abdomen is variable but usually widened at middle position of abdomen and tapered distally. Posterior of abdomen contains 5 short spinous appendages.
Zygoptera: They have three leaf-like gills at end of abdomen. Body is long and slender. Posterior of abdomen comprises 2 or 3 caudal appendages.

Ecological Role: Odonates nymphs are used as indicators in ecological assessments of lentic and lotic water bodies. Some odonates such as Aeshnidae are used as food in many parts of Asia including China. They are regarded as beneficial to human kinds because they prey on small invertebrates like mosquitoes.

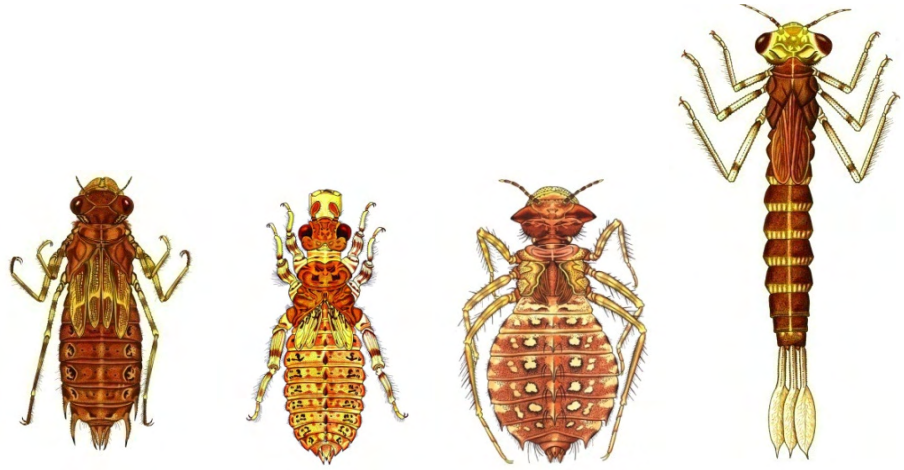

Figure 10. Odonata: Libellulidae (a), Gomphidae (b), Corduliidae (c), Coenagrionidae (d).Image © H Nesemann.

Key

A. Body slender, head wider than thorax; abdomen terminating in three long caudal leaf- or sac-like gills……..……..……..... Zygoptera (Damselflies)
B. Body short and stout; head narrower than thorax; abdomen lacking caudal gills but terminating in five short pointed appendages……..… Anisoptera (Dragonflies)

Zygoptera (Damselflies)

1. Caudal gills with a thickened, dark proximal portion and a thin, lighter distal portion; one premental seta on either side of the midline of the mentum Protoneuridae
- Caudal gills may not have clear proximal and distal portions; premental seta usually more than one on either side of the midline of the mentum…..... 2
2. Caudal gills long (approximately the same length as the abdomen); third segment of antenna longer than the second ..... Platycnemididae
- Caudal gills shorter than the abdomen; third segment of antenna shorter than the second; 3-5 premental setae on either side of the midline of the mentum ..... Coenagrionidae

Anisoptera (Dragonflies)

1. Prementum and palpal lobes nearly flat and without setae..... 2
- Prementum or palpal lobes are mask- or bowl-shaped and setae usually present ... 3
2. Antennae four-segmented; 3rd segment often large; prementum more or less quadrate; anterior margin of prementum without a cleft:

A. Elongated abdomen; burrowing in substrate ……. Gomphidae
B. More or less circular and widened abdomen, climbing on macrophyte ……..……... Lindeniinae

- Antennae slender, six or seven-segmented and are similar; prementum widest in the distal portion and narrowing towards the posterior part; anterior margin of prementum without a cleft ..... Aeshnidae

3. Cerci relatively longer than half of paraprocts ……..... Corduliidae
- Cerci shorter or nearly equal to half of paraprocts..... Libellulidae

5.2.7 Megaloptera (Dobsonflies and Alderflies)

Habits And Habitats: The Megalopteran larvae inhabit on the bottom of clear, cool freshwater streams and lakes. Habitat preferences include cobbles and stones. Only two families: Corydalidaeand Sialidae are known. They are active predators and feed on smaller aquatic invertebrates.

Distinguishing Characteristics: Abdominal segments I-VIII bear a pair of two segmented lateral filaments which are used as respiratory organs. Additional abdominal spiracles are present at each lateral side. Two prolegs with terminal hooks are present at end of the abdomen in Corydalidae. Sialidae possesses only one proleg with terminal hook at the end of abdomen.

Ecological roles: Their relatively larger body size than other macroinvertebrates make them good food for fishes.

Figure 11. Megaloptera: Corydalidae
(© 2011 Robert G Henricks)

Key to families

1. Abdomen terminates with two pairs of hooks; lateral filaments present from abdominal segments 1- 8 …….. .Corydalidae
- Abdomen terminates with a long median filament; lateral filaments present from abdominal segments 1- 7 ……...…. Sialidae

5.2.8 Decapoda (Crabs and Shrimps)

Habits And Habitats: The order Decapoda comprises of shrimpsand crabs. They inhabit in ponds, lakes, streams and rivers.
Shrimps occupy in wide range of freshwater habitats from torrential streams, larger rivers to ponds and lakes. Freshwater shrimps may be scavengers or predators.
Some freshwater crabs (e.g., sinopotamids) live entirely in water and others like potamids and parathelphusids are amphibious and feed in water and on land. They are omnivores and feed on algae, molluscs, worms, other crustaceans, fungi, bacteria and detritus.

Distinguishing Characteristics: Decapoda have five pairs of appendages (10 legs or peraeopods), of which the first (in crabs) and often the second (in shrimps) are chelate. Body consists of well-developed carapace that covers the head and thorax. Mouthparts contain front 3 pairs of appendages.

Ecological Roles: Decapodes have great economic importance to humans. They are prepared and eaten as a dish all over the world. They are used as pets in aquarium.

Key to Shrimps

1. Mandibular palp with a single terminal lobe; male abdomen triangular shaped ……..…….. Potamidae
- Mandibular palp with bilobed terminal part; male abdomen “T” shaped …… Parathephusidae
-

Key to Crabs

1. Rostrum consisting irregularly spaced spines; second pair of chelate limbs often longer than first …….……..……...… Palaemonidae
- Rostrum consisting regularly spaced spines; second and first chelate limbs approximately the same size …… Atyidae

5.2.9 Amphipoda (Scuds)

Habits And Habitats: Amphiopods are generally restricted to northern latitudes and higher altitudes (temperate regions). They inhabit in the shallow regions of streams, springs, lakes, ponds and marshes. They are commonly found in overhanging vegetation, root-mats and around cobble. They are detrital feeders and feed on fine organic matter.

Distinguishing Characteristics: They have laterally compressed body and C-shaped body at rest stage. Head consists of two pairs of antennae that are nearly equal length on cephalothorax. They have 7 pairs of walking legs and 6 pairs of appendages on ventral side of abdomen.

Ecological Roles: Amphipods are important food sources for fish and macroinvertebrate predators. However, they are of little importance to Asian tropical streams because of their restricted distributions.

Figure 13. Amphipoda: Gammaridae
Image © RDT Shah.

5.2.10 Platyhelminthes (Flatworms)

Habits And Habitats: The free living flatworms include Turbellaria (Planarian). They inhabit on the surfaces of rocks and wood in slowly flowing water but they can be found in lakes, ponds, streams, springs, and temporary water bodies.

Distinguishing Characteristics: They have flattened body. Body is not segmented and consists of two eyespots at anterior end.

Ecological Roles: Organically enriched water bodies enhance high number of Planarians. Therefore, they are used in ecological assessment of water bodies

Figure 14. Platyhelminthes (Dugesiidae)

Key to families

1. Anterior end triangular; one pair of large eyes present……..…… Dugesiidae
2. Anterior end truncate, one pair or many pairs of small eyes present …. Planariidae

5.2.11 Hirudinea (Leeches)

Habits And Habitats: Leeches live in standing waters such as marshes, pond and lake margins, and in the pools of streams and rivers. Some species inhabit in fast flowing waters. They are commonly found on aquatic vegetation, attached to prey, or on stones. Most leeches are parasites to other animals and feed on host organisms (e.g., fishes, frogs, birds and mammals) and some are scavengers.

Distinguishing Characteristics: Leeches have flattened body with visible rings or annuli. Eyespots are present dorsally at anterior end. Anterior and posterior ends comprise suckers. Head is at the narrower end of the body.

Ecological Roles: Some leeches have medicinal values.

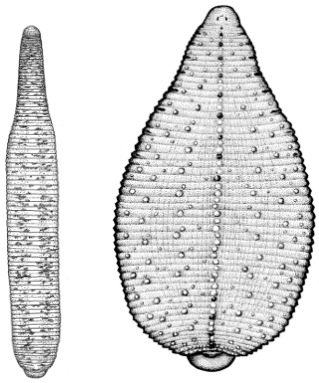

Figure 15. Hirudinea: Salifidae (left), Glossiphoniidae (right). Image © H Nesemann.

Key to families

1. Mouth small pore; a protrusile muscular proboscis present; teeth or jaw absent; body strongly flattened; usually <50 mm long……. order: Rhynchobdellida ..2
- Mouth large; proboscis absent; jaws or teeth may be present; body not strongly flattened; may reach > 80 mm long …… order.. Arhynchobdellida… 3
2. Anterior sucker not distinct from the rest of the body; eyes (one, two, three, or four) present……..… …… Glossiphoniidae
3. Five pairs of eyes arranged in an arch on annulin1-8; jaws may be present …… .Hirudinidae
- Three pairs of eyes variously arranged but never in a regular arch… Erpobdellidae

5.2.12 Oligochaeta (Worms)

Habits And Habitats: Oligochaeta are commonly found in lakes, ponds, marshes and stream pools. Some small kinds are found in the swift areas of streams. They are most commonly found in soft sediments e.g., mud and detritus. They feed on fine organic particulate materials.

Distinguishing Characteristics: Body is elongated and worm-like. Body is divided into many visible segments. Most segments have bundles of chaetae or seate.

Ecological Roles: Most oligochaetes are tolerant to organically polluted water. They are used in ecological assessment of water bodies. Oligocaetes such as Tubificidae are extremely tolerant to polluted water with very low dissolved oxygen levels.

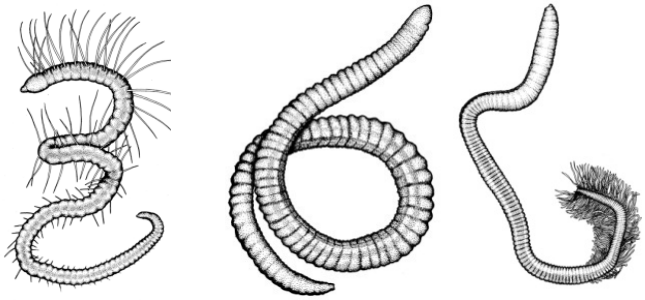

Figure 16. Oligochaeta: Lumbriculidae (a), Naididae (b), Tubificidae (c).Image © H Nesemann.

Key to families

1. Chaetae usually paired; if not, simple and pointed……..……2
- Chaetae more than two per bundle; usually bifid…….……3
2. Chaetae paired and bifid; if simple and pointed then in form of nodules; relatively large worms. …… Lumbriculidae
- Chaetae simple and straight; relatively small and thin worms……... Enchytraeidae
3. Worm usually 1- <10mm long; hair like chaetae in bundle which are made up of simple or bifid chaetae present mainly in the posterior segments… . Naididae
- Worm usually >10 mm long; dorsal chaetae normally present from II segment …… ….Tubificidae

5.2.13 Mollusca (Snails, Mussel and Clams)

Gastropoda (Snails)

Habits And Habitats: Gastropods inhabit in diverse water-bodies including streams, rivers, ponds, lakes, marshes and swamps. They occur on rocks, vegetation, silt, detritus and sand under water. They feed on algaeand aquatic plants, and play a vital role in the processing of detritus and fine organic matters.

Distinguishing characteristics: Body is enclosed by single shell. Shell is usually coiled. Snails can be divided into two groups (Prosobranchia and Pulmonata) depending on how they breathe. Prosobranchia groups use gills to obtain dissolved oxygen from the water and Pulmonata groups use lung-like structure to take oxygen from atmosphere.

Ecological Roles: Lymnaeidae and Planorbidaeact as intermediate host of trematodes which infect people and domestic animals. Pulmonta groups are taken as food by local people across the globe.

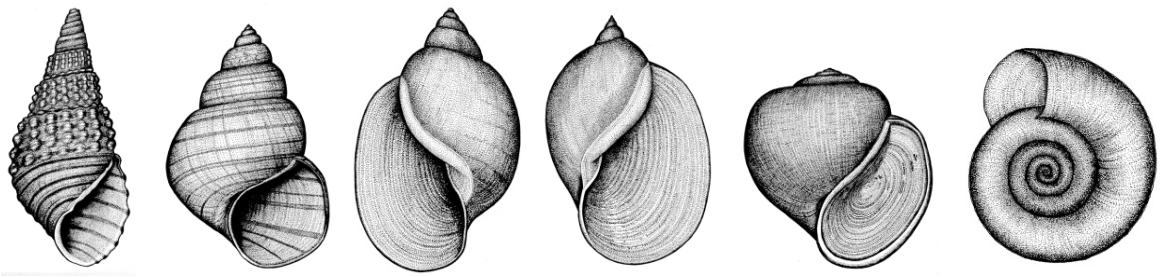

Figure 17. Gasstropoda: (left to right) Ampullariidae, Lymnaeidae, Physidae, Viviparidae, Thiaridae, Planorbidae. Image © H Nesemann.

Key: Order Prosobranchia

1. Shell globose or oval; spire usually depressed; operculum elongated ……..… Ampullariidae
- Shell in variable shape; spire not depressed …… 2
2. Shell with very weakly spiral ridges; with spiral colour band…… Viviparidae
- Shell with strong or transverse sculpture …… 3
3. Shell turreted shape; shell medium to large; operculum paucispiral or multispira …… ……..….. Thiaridae
- Shell not turreted shape; shell elongate; without spiral colour bands ….. Bithyniidae

Key: Order Pulmonata

1. Shell coiled in one plane ……..…… Planorbidae
- Shell elongated and coiled …….. 2
2. Shell coiled to left …… Physidae
- Shell coiled to right …… Lymnaeidae

Bivalvia (Mussels and Clams)

Habits And Habitats: Bivalves can be found in ponds, lakes, reservoirs, streams and rivers. They are highly diverse in large rivers and floodplain lakes. Some bivalves are attached to rocks but most bivalves burrow in stable gravels, sands and muddy substrates. They are filter feeders.

Distinguishing Characteristics: Body is covered by two shells arranged opposite of each other which are hinged dorsally by an elastic ligament. Each shell has teeth on its inner margins at the umbo (adjacent to hinge).

Ecological Roles: They are hosts for many aquatic invertebrates such as water mites, glosiiphoniid leeches. The shells are used for ornaments.

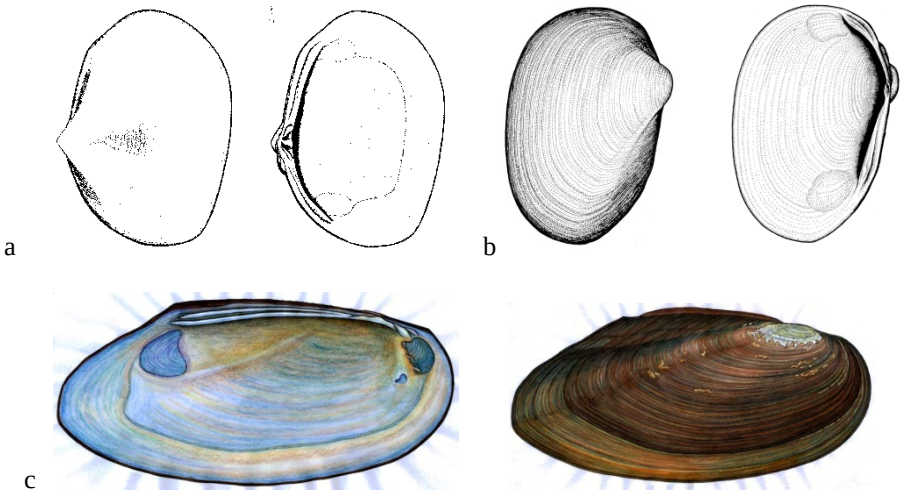

Figure 18. Bivalvia: Corbiculidae outer and inner view (a), Sphaeriidaeouter and inner view (b), Unionidaeouter and inner view (c).Image © H Nesemann.

Key to families

1. Shell relatively small (shell length <35 mm) and not elongated; internal shell surface not nacreous ……. …… ..2
- Shell relatively large (shell length >30 mm) and elongated; internal shell surface nacreous …… 3
2. Shell length >12 mm; with yellowish brown or dark olive-green or black …… Corbiculidae
- Shell length <12 mm; brown or cream colour shell …… Sphaeriidae
3. Shell length <70 mm ……..…… Amblemidae
- Shell relatively longer than Amblemidae; glochidia brooded in outer demibranchs; fusion of posterior mantle margins not extensive; Shell solid with brown or dark brownish periostracum …… …… Unionidae

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Annex I: Field Protocol for Lentic ecosystem (Source: Shah et al. 2011)

Investigator: _____		Date: _____	Time: _____
Name of wetland: _____		Sample code: _____	
Latitude: _____	Longitude: _____	Altitude: _____	
Hydrological characteristics		Hydrological classification	
Inlet [l/s]: _____ No. of inlet channel: _____		[ ] Periodic (regularly): summer dry winter dry	
Outlet [l/s]: _____ No. of inlet channel: _____		[ ] Episodic (non predictable): Permanent	
Feeding system			
[ ] Spring fed [ ] Snow fed [ ] Glacier fed [ ] Rain fed (monsoon)			
Morphological characterisation at sampling site			
Distance from bank: _____	Shading at zenith: _____	Removal of mineral bed materials: _____	
Mean depth: _____	Width of riparian vegetation: _____		
Hydro-morphological impact at sampling site			
Bank fixation: ____Concrete ____Stones ____Wood ____Other material ____No bank fixation			
Bedfixation: ____Concrete ____Stones ____Wood ____Other material ____No bank fixation			
Water abstraction: ____yes ____no	Purpose of water abstraction:_____		
Mineral habitat at sampling site		Biotic habitat at sampling site	
Clay:	Gravel:	Algae:	Submerged macrophytes:
Silt:	Cobble:	Emergent macrophytes:	
Bolder:		Living parts of terrestrial parts:	
Bed material:		Organic/inorganic debris:	
Signs of pollution at sampling site			
Source Pollution:		Non-source pollution:	
Eutrophication:		Mining:	
Morphometric and hydrological characteristics at sampling site			
Bed visible: ___Entirely ___Partly ___No		Water level: ___Low ___Medium ___High ___Artificial	
Water uses: _____Fisheries _____Recreation _____ Drinking water _____Cattle watering place			
_____Washing/bathing. Other (specify): _____			
Physical/chemical characteristics and measurements at sampling site			

Water color:		Odours:	Foam: yes/no	pH:
Conductivity [µS/cm]:		Turbidity: yes/no	Oxygen content [mg/l]:	
Temperature:	Alkalinity:	Total nitrogen:	Total phosphorus:	
Ammonium:	Nitrate:	Nitrite:	Ortho-phosphorus:	
Chloride:	Free $CO_{2:}$	Chlorophyll a:	BOD_5:	

Sampling method description		Sketch of sampling site _____ Photo no.:
Sampling technique used:		
Replicate number:		
Mesh size of net used:		
Size of sampler:		
Benthic taxa group (record no. of family)		
Ephemeroptera:	Lepidoptera:	
Trichoptera:	Turbellaria:	
Coleoptera:	Oligochaeta:	
Odonata:	Leeches:	
Diptera:	Gastropods:	
Heteroptera:	Bivalves:	
Malacostraca:	Others:	
Water mites:		

Annex II: Transformation scale with description and colour code for water quality classification (*modified from* Sharma and Moog 2005).

Biotic Index for Midland	Biotic Index for Lowland	Water Quality Class	Description	Colour code
6.51-10.00	6.00-10.00	I high	Non to slightly polluted	Blue
5.51-6.50	5.00-5.99	II good	Moderately polluted	Green
4.51-5.50	4.00-4.99	III fair	Critically polluted	Yellow
3.51-4.50	2.50-3.99	IV poor	Heavily polluted	Orange
1.00-3.50	1.00-2.49	V bad	Extremely polluted	Red

RAPID ASSESSMENT OF BIODIVERSITY

WATERFOWL AND HERPETOFAUNA

Nazneen Zehra

INTRODUCTION

Wetlands are known as "biological super systems" as they produce great volume of food that supports a remarkable level of biodiversity. The combination of shallow water, high levels of nutrients, and high primary productivity is ideal for the development of organisms that form the essential base of our planets food web. India is home for about 1340 bird species of which 310 species are known to be dependent on wetlands (Kumar et al. 2005). In a total, herpetofauna is representing ca. 11% of total world population is constituted by combination of ca. 475 species of reptiles includes 31 turtle species, 186 lizards, 3 crocodile species, and 255 species of snakes. Amphibians are enlisted 215 includes all living order such as salamanders, caecilians, frogs and toads. According to IUCN Red Data list (2012), six wetland birds and 19 reptiles and amphibians are recognised critically endangered. Besides, wetlands play vital role in other ecosystem services such as flood control, aquifer recharge, nutrient absorption and erosion control (Kumar and Gupta 2009). Wetlands, in India cover an area of ca. 15.62 m ha. Of which inland wetlands accounted for 10.56 m ha. (Panigrahy et al. 2012), but today these ecosystems are facing tremendous anthropogenic pressures, which can greatly influence the structure of existing water based biodiversity as well as human dependence (Desta et al. 2012). Wetlands received attention only after signing of intergovernmental treaty "RAMSAR convention (1917)" with concern of conservation of wetland habitat particularly for migratory waterbirds, whereas inland biodiversity perceived importance only in COP4 (1998) in Bratislava (Gopal 2005). So far as the wetland importance is concerned, few publications list the group of species, specifically herpetofauna from Western Ghats and north east regions (Vasudevan 2008, Das et al. 2009, Pawar 1999) and wetland birds from all over India (Urfi et al. 2005). There is a significant variation in conservation status, from widespread and locally abundant to severely restricted species. The over-riding cause of declines has been (and in many cases continues to be) the loss, modification and fragmentation of habitat through agricultural intensification and development. Other factors include natural succession leading to unsuitable habitat structure (especially shading), introduction of invasive flora and fauna, fires, inappropriate habitat management, effects of public access, and persecution. The type and significance of the decline factor varies across the species, and in many cases several factors act together synergistically.

KEYS TO IDENTIFICATION OF MAJOR TAXA

The observer should be aware or trained to identify all of the focal species potentially trapped in the study area. It can be done by studying field guides, and by training with an experienced field biologist. If the observer cannot positively identify an animal, there should be made one descriptive note about species morphological unique features and habitat type and photographed for further inquiry. Start bird identification by identifying general groups of birds (whose members shares certain similarities), when observations improved, familiarize yourself with the basic morphological features. For that draw a quick sketch that allows you to point to different parts of the bird and label colours or features (Figure 1a). For example, point to the top of the head and write down any colouring, feather colour pattern i.e. primary, secondary and tertiary, eyes and bill colour. Any unique identity if you can recognize. Having the sketch will help you think of all the different parts of the bird to describe. Only after writing down all the characters proceed to consult a field guide (Ali & Ripley 1983, Grimmett et al. 1999).

In case of herpetofauna, the animals trapped alive should be identified, marked and numerated soon then release them immediately.

Identification of Species with Limbs

During identification of limbed reptiles and amphibians, first record the array and trap number in which species is trapped. Record species morphological feature, colour pattern, measure length and weight. Species age can also be observed in categories as juvenile and adult. Species marking can be done by assigning number to the toes (frogs, toads, lizards, salamander etc.). Numbering should be done in clockwise pattern beginning from left front foot (Fisher et al. 2008) (Figure 1b). Turtles can be individually marked by making notches on the edges of the marginal scutes or by painting the shells, depending on the level of permanency needed in the study (Fisher et al. 2008).

Identification of Other Limbless Species

During identification first follow the predefined identification instructions as per the possibility and then proceed to next level in which snakes (except venomous snakes) can be marked by scale-clipping at ventral side of tail, posterior to the vent or anal plate. Scales are assigned numbers by looking at the individual from the bottom or ventral side with the head up in the 12 o'clock position (Figure 1b). All species are inspected for markings upon capture. Marked individuals are noted as recaptures. A newly captured individual is assigned a new number in sequence. The number is marked as used on the scale-clip chart and later removed. As per the safety instructions venomous snakes should not be measured and weighed, these measurements can be assumed in approximate without handling the individual. In case of pitfall or funnel trapping, venomous snakes can be removed carefully by using a snake stick if they are not large enough to get out themselves or by carefully removing one end of the trap, tipping the snake out a few meters away from the array. Tadpole identification is difficult and requires knowledge of unique combinations of the vent, spiracle, and eye positions on the body, oral disc morphology, and dentition. Egg mass identification can be done reliably to genus; species-level. Identification requires more experience and typically knowledge of breeding species and their phenology at a site.

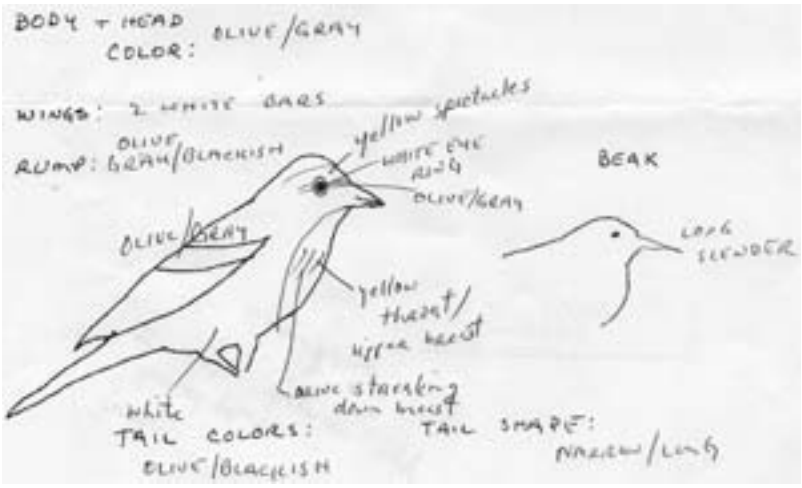

Figure 1a. Identification sketch of a bird.
(http://feederwatch.org/wp-content/uploads/2013/07/SketchPinwar253500_04.jpg)

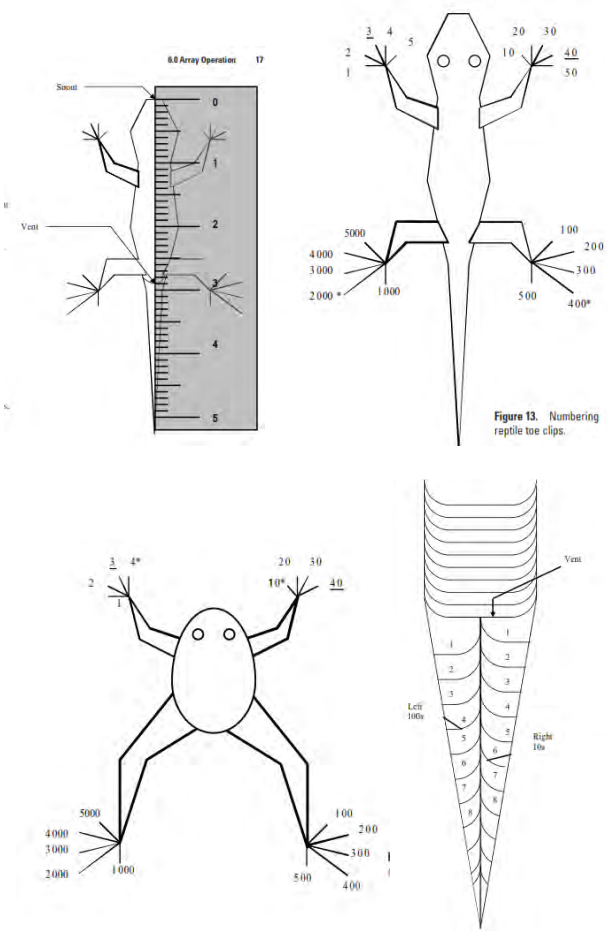

Figure 1b.Identification, numeration and marking of herpetofauna.

Few key features which can help in identification are as follows:

- Reptiles are almost exclusively diurnal whilst amphibians are largely nocturnal;
- Amphibians lay jelly coated eggs in water and have a larval stage, while reptiles may give birth to live young or lay eggs on land and have no larval stage;
- Reptiles have impermeable, scaly skin while amphibians have moist, glandular, permeable skins; amphibians typically have larger numbers of young, with higher early stage mortality and more unpredictable survival than reptiles.
- The key features common to both amphibians and reptiles is ectothermy (the dependence on external sources of heat to allow activity, because of an inability to raise body temperatures via internal means), small size, lack of truly social behaviour, and relatively oddest dispersal abilities.

METHODOLOGY

Biodiversity monitoring of wetlands is a challenging task and require combination of methods to obtain robust findings. Herpetofauna (reptiles & amphibians) and birds frequently inhabit wetlands and adjacent uplands. Because if amphibians complete initial stage (larva) of their life cycle in water then juvenile and adult stages in terrestrial ecosystem and few reptiles (i.e. order: Chelonia, Squamata, Crocodilia) spend much time of their life cycle totally in water. The monitoring of these species can be done using a combination of aquatic and terrestrial methods which again have categorised in active (those that attract animals but require an observer to actively capture them at the moment of the census i.e. Coverboard, PVC Pipes, Litter Bags) and passive (those, actually trap animals,

accumulating captures on their own over time i.e. Drift fence, ACM) methods (Willson and Gibbons 2009, Thompson 1991). Wetland birds can be monitored using round count, area search, and areal count (Koskimies and Poysa 1989, Urfi et al. 2005, USFWS 2014). For these groups of species methods can be opted based on the monitoring object and species of interest. I am describing here few methods, if these methods incorporate into a robust sampling design, could provide a robust database.

METHODS FOR WATERFOWL

Population Status and Trend

The round count, area search are most commonly used methods to assess population status and trends at small sample area (ca. 10–16 ha). While aerial count is useful at vary large landscape.

Area search or direct count (Weller 1999) sampling involves viewing a defined area over for a specified short duration (i.e., 3–5 min) with binoculars or spotting scope and counting the number of individuals present in the area by species. It is appropriate for the parts of wetlands where visibility is unobstructed.

In round count (count birds from a boat or when walking round the water body) and point count (count birds from 1-10 fixed points), start the monitoring in areas where there are few birds and end it where the number are highest. During the monitoring pay attention to bays, mouths of ditches and edges etc. as preferred by waterfowls. The monitoring should be conducted during the 6–8 week period when most water birds migrate (i.e., Summer and Winter). Monitored sites are revisited every 7–10 days during this period.

In other methods, waterfowl recruitment (counting of broods and nests) is there to monitor annual changes in breeding success. The practice includes systematic search of wetlands, once broods or nests are located, they are monitored for activity every 3–5 days. Breeding success can be calculated using number of successful nests divided by the total number of nests monitored (i.e., raw nest success), or by less biased methods involving calculation of the number of nest survival days using the Mayfield method (Mayfield 1975).

Although birds banding and radio-telemetry also (Javed et al. 2003, Kumar et al. 2010) help in population estimation by contributing data on mortality rates, tracking of birds movements, and habitat use during all stages of the annual cycle (i.e., breeding, migration, and wintering).

METHODS FOR HERPETOFAUNA

Egg Mass & Larvae Count

Egg mass and larvae counts can be used as an index of adult population size and reproductive efforts (Paton and Harris 2010). Most egg masses deposit in the littoral zone of wetlands in water that is <60 cm, thus during practice observer should focus in areas close to the water edges. Some species prefer to lay egg masses amongst vegetation (e.g., *Pseudacris spp.*), whereas other species prefer more open water (e.g., *Lithobates catesbeianus*), thus counts can be performed using dip net sampling (it is useful for shallow water) or seine net sampling (it is useful for deeper water) at least twice per year (i.e., spring and summer) to incorporate breeding phenology (Paton and Harris 2010). During practice, pull the seine nets (0.48-cm mesh) over a specified distance and it is most effective if emergent vegetation is absent. In dip netting (with a large opening e.g., 40 × 40 cm and deep net >50 cm with fine mesh <0.25 cm) plunge it down into the water including the leaf litter and quickly scooped upward (Figure 2). It can be performed at sampling points along transects that traverse the elevational gradient of the wetland or in random locations within the emergent vegetation zone. Counts can be performed along a transect or within a designated area, and record search time and number of observers to standardize relative abundance estimates which can be done by dividing number of samples (egg masses or larvae) counted per species by the collective minutes searched for all

observers then divide this quotient by the number of observers. This estimate can be compared among years and sites if egg mass detectability is similar.

Figure 2. Dip net and Seine net sampling.

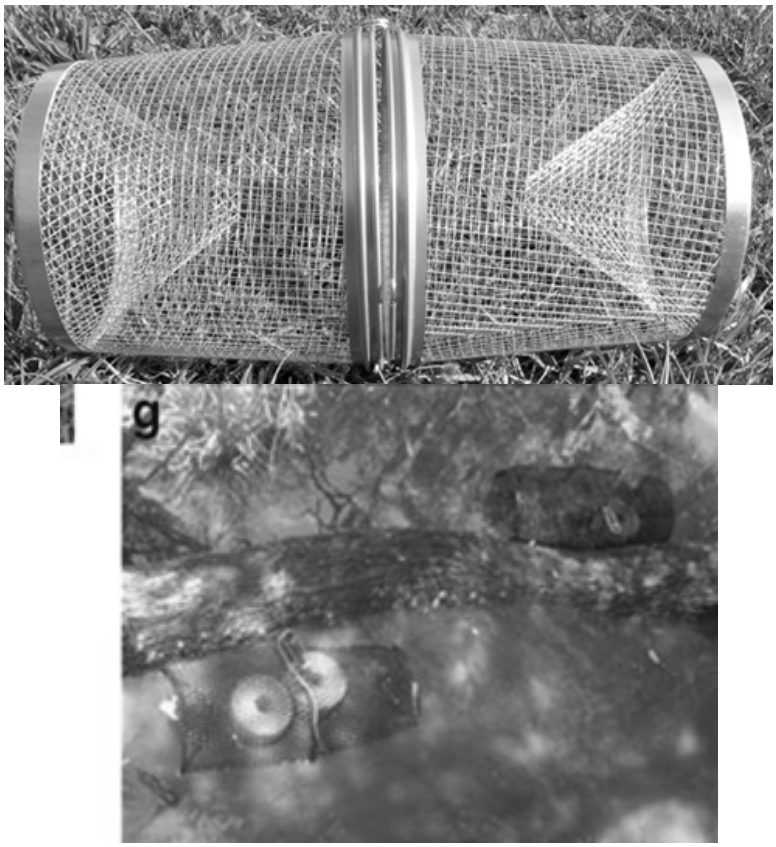

Figure 3. Minnow trap sampling.

In other methods, trap sampling (minnow and funnel traps; Figure 3) and enclosure samplers are there. The most common type of trap used to capture amphibian larvae is a minnow trap, which contains two opposing funnels that taper inward. Larvae are naturally directed into the tunnel, and after passing through a small opening are unable to find the opening again. During practice, place minnow traps in shallow water with at least 10% of it exposed to provide air and check it at every 12–24 hr. If placing traps in deep water zones of a wetland, it should be tethered to a permanent structure

(e.g., tree or stake at the edge of the wetland) to prevent the trap from sinking or floating away, and to facilitate relocation.

Enclosure samplers are either rectangular (box-type) or circular, and are designed to enclose a designated area for sampling (Mullins et al. 2004, Skelly and Richardson 2009). In practice, place enclosures into water with about 5cm of the bottom sunk into the substrate and the contents netted (Figure 4). Nets should be small (20 × 13 cm) with fine-mesh and a sturdy handle. Dip the nets repeatedly through the entire water column and surface area for a minimum of ten times until it results in no captures. Later on, place captured larvae in a holding container to identify and enumerate them. Similar to the other methods, enclosures can also be used either randomly or at transect in the wetland.

Figure 4. Enclosure sampling.

Visual encounter surveys can be conducted to sample aquatic reptiles in wetland ecosystems. It is commonly used to detect species during sun basking (e.g., turtles, semi-aquatic snakes, and crocodiles) (Figure. 5). Sampling should be performed at a distance (20m or greater) to prevent disturbance. It is recommended establishing viewing stations systematically around a wetland. Binoculars or a spotting scope should be used to aid in detection and identification of species and individuals. Basking reptiles are frequently detected on emergent structures (e.g., logs and stumps) and along the banks of wetlands or rivers that are devoid of vegetation. Surveys should be carried out late morning through mid-day (10:00–15:00). Nocturnal surveys from watercrafts with a bright spotlight are useful for detecting crocodiles. It provides estimate of species occurrence or relative abundance. Mark-re-sight techniques with highly visible marks can also be used in this regard.

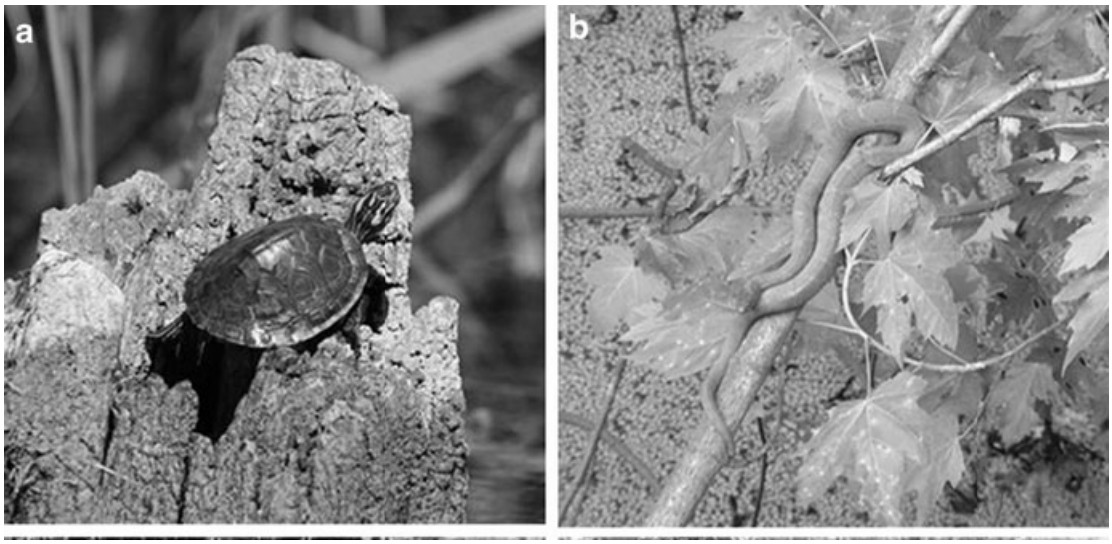

Figure 5. Visual encounter survey.

Terrestrial Drift fence along with Pitfalls and PVC pipes

This is a most common method to collect information on adult population size and processes (e.g., survival, dispersal). During the practice, place drift fence at adjacent upland of a wetland. The bottom of the fence should be buried to prevent amphibians from crawling underneath. The drift fence placement is standardized at 10 m above the expected high waterline and parallel to the wetland (Figure 6). It can be used on the focused area partially or completely and in segment or arrays Pitfalls should be placed adjacent to the drift fence typically every 10 m and at the ends of fence sections to capture amphibians that intercept the fence. Standard placement is two opposing pitfalls; one on each side of the fence but an alternative design can also be used as one pitfall on alternating sides of the fence at every 5 m (Labanowski and Lowin 2011). The use of only pitfall is biased for most amphibian communities as many species of frogs (Hylidae & *Lithobates catesbeianus*) species can climb out. Then PVC pipes are of important use along with fence for effective capturing. When pitfalls are not covered, they should be checked at least every 24h, and recommended to keep opened at least 2 days per week. Afterwards, identify, numerate, marked uniquely, and release on the opposite side of the fence that they were captured.

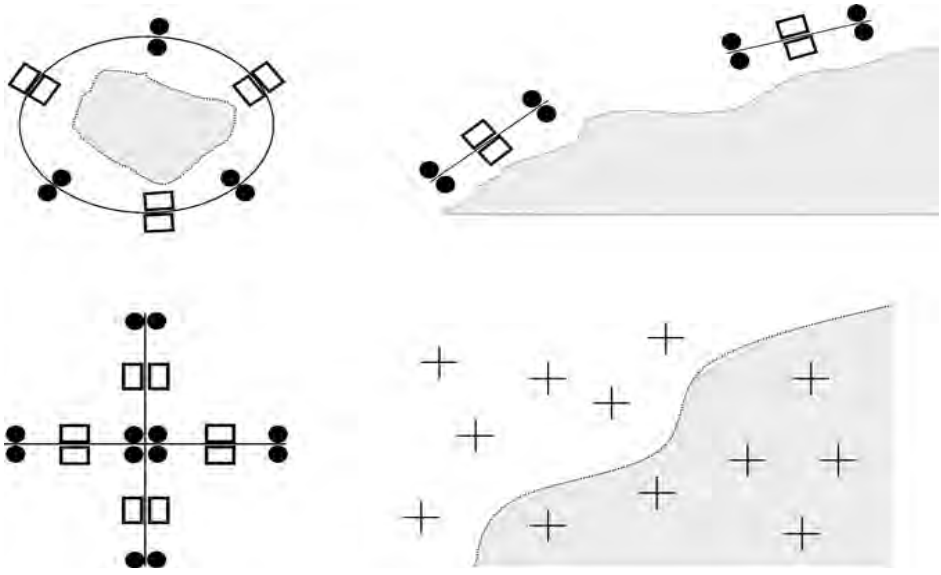

Figure 6. Drift fencing along with pitfalls.

Artificial cover objects (leaf, wooden board, tin, litter bags etc.) can be used as a technique to supplement species detection as create moist microhabitat during the day. The odds of catching amphibians are often greater under wood objects, whereas reptile captures tend to be greater under tin (Figure 7). During practice, objects should be deployed for at least one month prior to sampling so that suitable microclimate conditions develop under the object. Searching during nights with rain can increase the likelihood of detecting individuals. Search can be done at three levels as level 1 ¼ counts of amphibians on the surface only, level 2 ¼ level 1 and amphibians detected under natural cover objects, and level 3 ¼ previous levels and intense searches through leaf litter and the interior of decaying logs. Level 2 is most commonly used because level 3 destroys amphibian habitat, and detection is low for level 1 except during rain events.

Adaptive cluster sampling (ACS) is an area-based search using plots or belt transects. Plots are typically 10×10 m or 25×25 m, with larger plot sizes used when amphibian densities are low (Khan et al. 2010) (Figure 8). The most common transect dimensions are 50 or 100 m in length and 1 or 2 m in width. Plots or transects can be randomly or systematically placed in a sampling area; adaptive cluster sampling is recommended for species that are uncommon or have a clustered distribution.

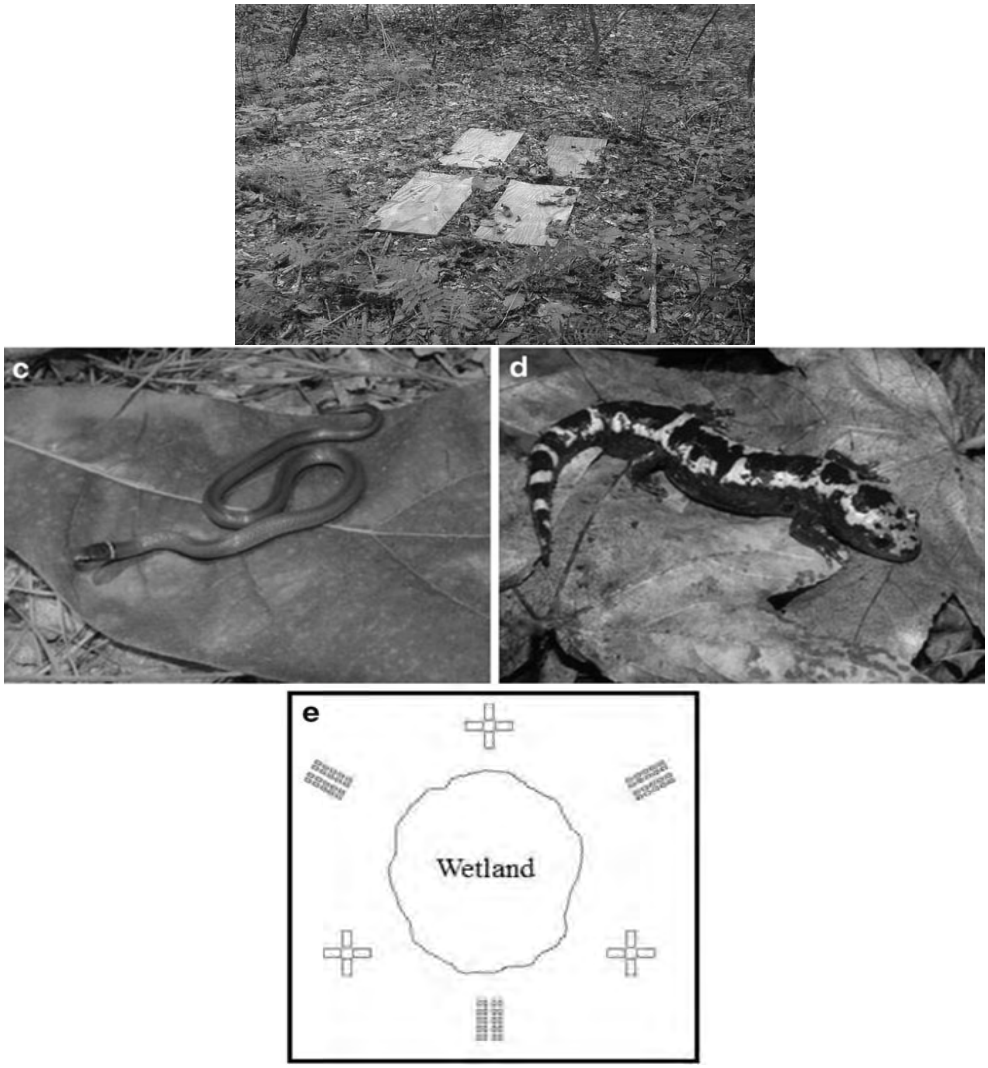

Figure 7. Artificial cover objects sampling

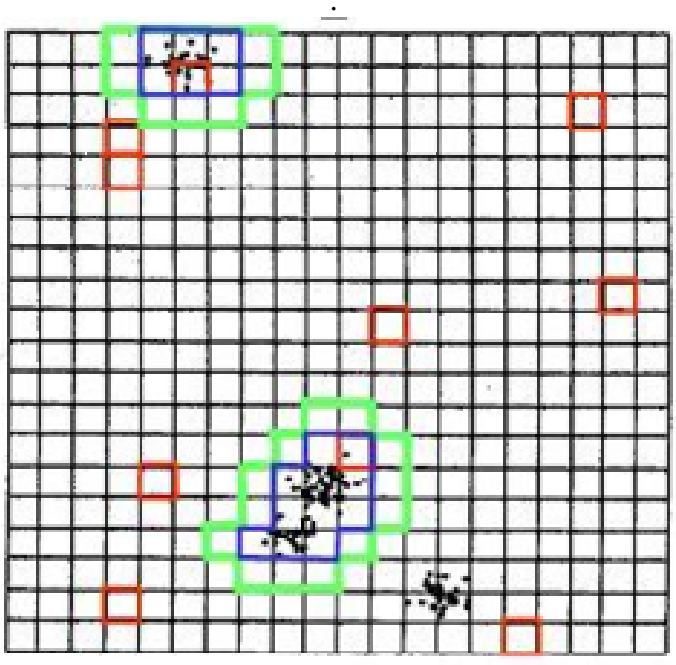

Figure 8. Adaptive Cluster Sampling

Call survey is the method to document probable occurrence using advertisement call. These calls are produced by adult males of most frog and toad species during breeding to attract females. Anuran calls are unique among species, and most species can be reliably identified with practice. Calls can be recorded by observers or automated recording devices by deploying them overnight. The protocol specifies to perform call survey between 30 min following official sunset and 0100 h (NAAMP, http://www.pwrc.usgs.gov/naamp/). Several studies suggest that 5 min is adequate to detect most breeding anurans (Burton et al. 2007). A call index can be maintained as: 1 - when calls from different males do not overlap, 2 - when calls overlap but individual males can be distinguished, and 3- when calls overlap and individual males are indistinguishable. Surveys should be performed at least once monthly from early spring through summer to encompass most of the anuran breeding season.

DATA ANALYSIS

The analysis can be done using Margalef's D Index, Shannon Wiener and Simpson's D Index. Recruitment data can be analyzed using Program MARK and the logistic exposure model which is similar to predictions using the Mayfield method (Rotella et al. 2004). Total bird count can be used to estimate abundance using software program WILDLIFE COUNTS (www.**wildlifecounts**.com).

ECOSYSTEM SERVICES OF WATERFOWL

Waterfowl are the most prominent organisms of which a fewspecies often occur in huge numbers and provide a spectacle that attracts humans from far off places to just watch them. As mentiomed earlier, in man cultures, waterfowl have been objects of reverence as well as exploitation since historical times. It was already pointed out earlier that waterfowl conservation laid the grounds for wetland protection and the Ramsar Convention, without even known scientifically their enormous contribution to the wetlands functioning which weredocumented later through studies worldwide. The waterfowl include taxa which have a very wide range of habitat requirements, feeding and breeding habits, and behavioural characteristics besides the long range migration by many of them. The role of waterfowl in structuring other bioic communities of wetlands was discussed by Marklund et al. (2002). Their contribution to even regulating climatechange by influencing methane emission was demonstrated by Bodelier et al. (2006). There is a very large bulk of published literature on the ecosystem services of different groups of waterfowl and these studies have been highlighted among ohers by Sekercioglu (2006), Whelan et al. (2008) and Wenny et al. (2011). Very recently, Green and Elmberg discussed the ecosystem services by water birds in some detail. A summary of these ecosystem services is given in Table 1, adapted and modified from Green and Elmberg (2014) which must be consulted for details and numerous references.

ROLE OF AMPHIBIANS AND REPTILES IN WETLANDS

(contributed by Prof. P.C. Bhattacharjee)

- Dispersal of food plants especially their seeds. Turtles play a major role in this.
- Subterranean species help aerate hard soil, allowing the access of air to the roots of the tree in rainforest/evergreen forest.
- Frogs are known as the agent of pest control in paddy. Often Paddy and wetland ecosystems are back to back in the floodplain context.
- Lizard like Indian Spiny-tailed (*Uromastyx hardwikii*) a significant predator of locusts
- Rodents and water bird populations are controlled by *Varanus* sp.
- Frogs and freshwater turtles are control agent of aquatic insects including mosquitoes and snail (disease vector).

Table 1. Ecosystem services of major groups of waterfowl
(modified from Green and Elmberg 2014)

Category	Ecosystem service	Waterbird taxon
Provisioning	Meat	Anatidae
	Down	Common eider, geese
	Feathers for clothing and ornaments	Anatidae, herons, others
	Grease for waterproofing	Geese
Supporting	Animal propagule dispersal	Anatidae, coots
	Plant propagule dispersal	Anatidae, shorebirds
	Nutrient cycling	Geese, cormorants
	Stimulating primary productivity	Geese
	Stimulating decomposition	Ducks
	Reduction of methane production	Swans
	Plant diversity	Anatidae
	Animal diversity	Anatidae, others
	Protection from predators	Geese
	Bioindicators of plants	Anatidae, coots
	Bioindicators of animals	Anatidae
	Bioindicators of nutrients/contaminants	Herons, grebes, ducks
Regulating	Pest control	Ducks
	Disease surveillance	Ducks
	Regime shifts of wetlands	Cormorants
Cultural	Recreational hunting	Anatidae
	Birdwatching	Geese
	Ecotourism	Geese
	Conservation flagships	Anatidae, flamingoes
	Art	Flamingoes, others

- Some tadpoles, being carnivorous, control population of other aquatic fauna as well as clean the dead animals tissues from water.
- Keystone species for an aquatic ecosystem like crocodileans (mugger, salt water crocodile and gharial) are top predators. Mugger has a wider food range which includes, fishes, reptiles, birds and mammals whereas gharials mostly feed upon fishes and crustaceans. They help to maintain the ecological balance in aquatic ecosystem.
- Turtles play a major role in cycling and retention of nutrients in wetlands. Turtles also help to release nutrients locked in the bottom of a wetland and thus help increase the productivity of a wetland. Softshell turtles are apex predators of the wetland ecosystem (where crocodilian do not occur), and also serve as scavengers.
- Amphibians are used for many kinds of both traditional and modern day medications.
- Amphibians are biological indicator. Super sensitive species are best indicator to ecosystem and environmental health.
- Aquatic frogs (living mostly in water) help control the balance in the wetland ecosystem by controlling the arthropod populations.
- Some snakes live in paddy fields and voraciously feed on rodents and thus, help in crop protection.
- The lizards of genus Varanus (4 species in India) are true wetland dependent species. They are prime scavenging reptiles helping in carrion control.

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RAPID ASSESSMENT OF BIODIVERSITY

WETLAND FISHES

Vikash Kumar and M.A. Hassan

INTRODUCTION

Taxonomy is the science of the description and classification of organisms, essential in theoretical and applied biology. The word taxonomy is derived from the Greek words *taxis* (=arrangements) and *nomos* (=law). Taxonomy and systematic have two main goals: (1). primarily of academic interest: the study of the diversity of living organisms and their phylogenetic relationships, and (2). immediate practical interest: inventories, surveys, documentation of biodiversity and the compilation of identification tool. For the proper management of natural resources, we need information on the number of species and their identification (Kottelat 2013).

Correct species identification is the basic starting point for any type of biological study, particularly on wild populations and it is important that each name applies to only a single species and that each species is known by a single name (Rainboth 1996). Specific rules have been established for recognizing, naming and classifying species to avoid redundant descriptions or the use of the same name for more than one species. Some people who fail to differentiate between two species by human eye often name them as cryptic species and molecular techniques and complex statistics are used to justify recognition of species. However, these so called cryptic species because no trained taxonomist ever had an opportunity to examine them (Kottelat 2013). The rapid and accurate characterization of species using morphological data is a critical constraint. To overcome this, species identification using molecular tools has been supplemented in many studies in present times (Chandra et al. 2012, Vinoth et sl. 2012). Species identification by DNA bar-coding is based on sequencing a short standardized genomic region of the target specimen and comparing this information to a sequence library from known species (Chandra et al. 2012). DNA bar-coding is an alternative to traditional taxonomic methods that could become a useful tool for coral reef conservation (Vinoth et al. 2012a,b).

The general interest about biodiversity conservation, the advances of internet and web pages, progress in molecular techniques, the development of statistics in phylogeny and a global perspective on taxonomy is giving some lights in taxonomy and it is becoming fashionable again. Identification, cataloguing, studies on the biology of the fishes, assessment and evaluation for their criteria has become inevitable for their conservation and sustainable utilization. The chapter gives the systematic approaches towards a classical fish taxonomy and aims at easy identification of freshwater fishes.

FISH SAMPLING FOR IDENTIFICATION

Collection of specimens

In order to collect the specimen one must have the knowledge of all possible geographic information of the surveyed place, including the distribution of various types of vegetation, altitudes, seasons, means of transport, lodging, etc. However, main emphasis should be given to the basin concept not to the political boundaries of the aquatic system. It is also necessary to examine the previous collection of the concerned group to know the various localities of the already collected materials. The collection should be made from all zones of water body, including surface, bottom, middle, upstream, midstream, and downstream.

Methods of collection:
There are numerous methods to collect fish specimens round the year. Fishes were collected using nets, traps, hooks and lines, electro-fishing equipment, hand picking, buying from local fisherman and from local markets, etc.

Data collection:
The specimens which are being collected must bear collection data. A specimen without such data is completely useless for a taxonomist. Thus, every specimen collected must be labeled having the following data:

Geographical location: Country, state, village, drainage basin, name of river, lake, etc. A fish taxonomist should have a rich knowledge of the drainage concept. Some fish are endemic to a particular basin and they are not found in another basin.

Date of collection: Date on which the specimen was collected

Name of the collector: Name of the fisherman, scientist, etc.

Coordinates: To be noted using GPS

Colour: Colour of the specimen in fresh should be noted.

Fixation and preservation:
The collected specimens should be first fixed in the preservatives (10% formalin or 70% ethanol) in a large container so that the specimen maintains its original shape after fixation. Preservation in tight containers distorts the shape. Fishes may also be preserved in buffered 10% formalin. Buffered formalin is prepared by adding three grams of borax per litre of 10% formalin solution. It retards the tissue shrinkage and prevents decalcification of the tissue. A general rule is maintained not to have more than 40% of biomass per container of formalin during fixation (Figure 1).

Figure. 1 Methods of fish preservation

After fixation the specimens are put in a glass stoppered bottles or screw capped jars with the snout pointing downwards and then filled with freshly prepared preservative. An incision should be made on the abdominal wall of the fish length 10-30 cm to one side of the mid-ventral line with a scalpel knife or scissor. And for the fish longer than 30 cm undiluted concentrated formalin is injected with the help of a hypodermic syringe throughout the abdomen (Figureure 1).

Tissue sampling:
Fresh tissue in the form of muscle, fins, etc. are collected from certain specimens and preserved in ethanol for molecular studies.

Morphometric and meristic characters

Morphometry refers to body proportions and meristics to counts. Measurements were made point to point on the left side of the specimen whenever possible, as shown below. Proportion of body parts are expressed in per cent of standard length and parts of head in head length.

Counts include fin rays: soft, hard, spinous, simple and branched; lateral line: longitudinal and transverse; predorsal scales, circumferential and circumpeduncular scales; branchiostegal rays, gill rakers, pharyngeal teeth, vertebrae, etc. counts and measurements usually follow Kottelat (2001) unless it is modified by different workers for particular genus or family for convenience. The values may be entered in a data sheet.

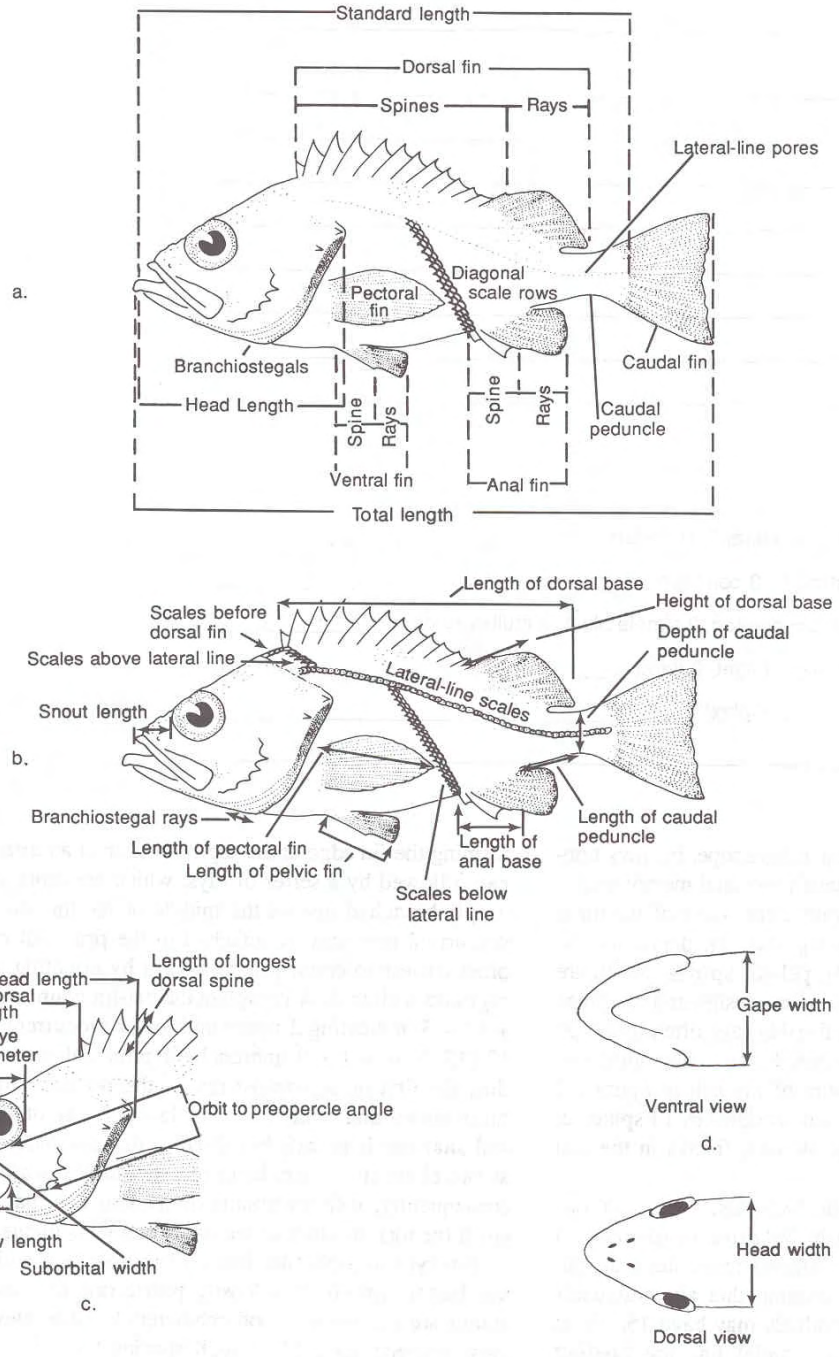


Figure 2. Morphology of fish (from Cailliet et al. 1986)

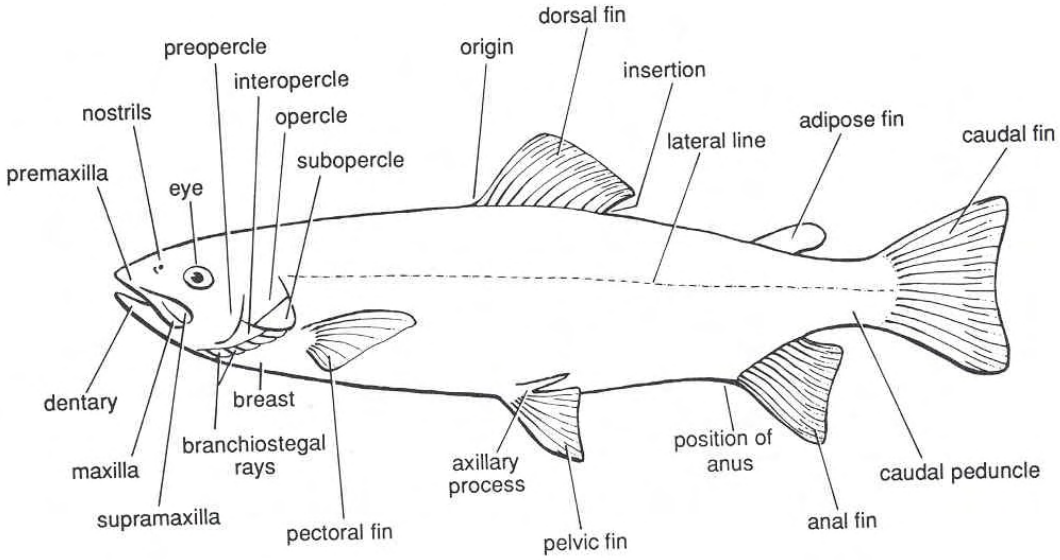


Figure 3. Morphology of fish (from Shreck and Moyle)

Counts	Common Name: / Species:
Dorsal fin elements	
Anal fin elements	
Pectoral fin elements	
Scales along the lateral line	
Branchiostegal rays	
Total gill rakers on first arch	

Measurements	Common Name: / Species:
Standard Length	
Body depth	
Caudal peduncle depth	
Predorsal length	
Length of dorsal base	
Length of anal base	
Height of dorsal fin	
Height of anal fin	
Length of pectoral fin	
Length of pelvic fin	
Length of longest dorsal spine	
Head length	
Head width	
Snout length	
Suborbital width	
Eye diameter	
Upper-jaw length	

Qualitative	Common Name: / Species:
Position of mouth: *Inferior, terminal, superior*	
Snout Profile: *Convex, concave, straight*	
Upper-jaw teeth shape: *Simple pointed, simple blunt, multicuspid*	
Shade of body background color: *Light, dark*	
Pattern of body color: *Plain, complex*	

Osteology

Osteological data were taken from cleared and Alzarin stained specimens. Disarticulations of bones, without damaging their structure, are done by treating the fresh specimens directly in 2% KOH solution without any preservative. The specimens are suspended in the solution for 5-7 days to facilitate the natural decomposition of muscle and ligaments. Addition of preservatives should be avoided since it caused hardening of these tissues, which result in the damage of the bones at their sutures at the time of manual separation. After this treatment, the bones are stained with Alzarin. The stained bones are preserved in glycerine solution or 10% formaldehyde buffer solution. Identification of bones was based on Prokofiev (2009, 2010). Rare specimens are not dissected. Instead radiographs may be taken for osteological studies.

Cataloguing of specimens

The entries usually followed in cataloguing are consecutive museum number, scientific name, locality, date and collector. The entire specimen collected from one locality or district by one expedition are catalogued together. The specimens are usually catalogued after they are identified at least up to genus level. The museum register must have at least the following information (Table 1).

Table 1: General format for cataloguing the fish specimens

Reg. No.	Zoological name	Family	Locality, latitude, longitude, etc.	Collector or donor	Date of collection	No. of specimen	Determined by	Date of entry	Remarks (whether holotype or paratype)
1									
2									
3									
4									

Comparison with the nearest congeners

The specimen under examination is compared with its congeners. Firstly, they are compared with the species from the same basin and then with the species of the same genus, the comparison is normally done with the type specimen from their respective type locality. The best means to identify the specimen is the direct comparison. When the literature on a species is not available, a specimen is compared with the already identified one. This approach is useful at any level. The type specimens are the most authentic at all. The original verbal descriptions of specimens are the permanent records of the attributes of a given species.

Data Sheet

Meristic counts (fin count, scale count), different body ratios, general body coloration, etc. are entered in a data sheet. The description is thus one of the most essential steps in the taxonomic studies. If a taxonomist described a new species, the description given by him will then serve as the basis to identify this new species for future workers. It includes diagnostic as well as those characters by which it can be differentiated from yet to be discovered species. Descriptions including meristic counts, different body ratios, etc. are written in a very elaborate manner. The colour of the specimen will be changed due to formalin preservation so a taxonomist should note the colour of the fish, spots, blotches, number and design of bands on the live fish itself and also after preservation.

Key: This is one of the most commonly used methods for identification. The new species is then studied and we see whether it fits into the available key of that particular genus, if not then we can easily prove that it is a new species.

Reporting

The species is reported by well-organized description. It normally includes original references based on which the species is identified and confirmed, records of materials examined, diagnosis, description, distribution etc. In case of new species, the registration number of new type species, size, locality of collection, collectors name, date of collection and museum where deposited should be mentioned.

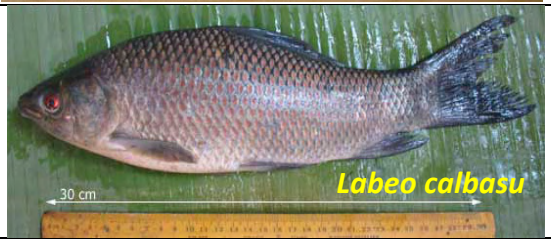
Diagnosis
Diagnosis includes only few characters by which the species in question can be easily separated from other similar to its nearest congeners.

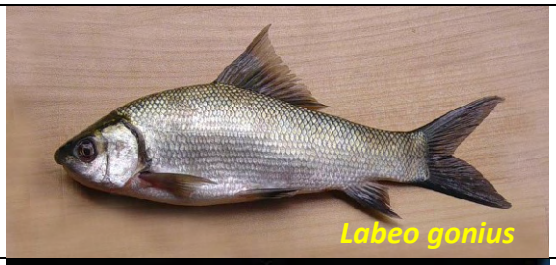
Systematic description
A systematic description means description of the species based on the observation. It includes descriptions of different body parts in respect of shape, position, counts and proportions. Details of osteology, if done, may also be reported. Colour description in fresh and preserved state is important. The description should be supported by tables and illustrations of the whole body and the important parts to show its diagnostic characters.

Common fishes of wetland and their identifying features

The wetland with diversified micro-ecosystems such as shallow macrophyte infested zone, deep clear zone, cyclic lentic and lotic phases provided habitat for a variety of fishes. For easy identification of commonly encountered wetland fishes, images along with their identifying features are given below.

Classification of the fishes based on order, family and other key identification features

Order: Cypriniformes Family: Cyprinidae	
Species	**Identifying featues**
	✓ Head is large with upturned mouth, with a prominent protruding lower jaw, ✓ No barbells, no jaw teeth but 3 pairs of pharyngeal teeth, ✓ Body deep, with depth 2.5 to 3 times in standard length and pectoral fins long, extending to pelvic fins, ✓ Dorsal soft rays 17-20; Anal spines 0; Anal soft rays 7 – 8 and lateral line with 40-43 scales.
	✓ Body bilaterally symmetrical and streamlined, its depth about equal to length of head, ✓ Body with cycloid scales, head without scales; snout blunt, often with pores; mouth broad, transverse, ✓ Upper lip entire and not continuous with lower lip, lower lip most indistinct, single pair of short rostral barbells, ✓ Pharyngeal teeth in three rows, 5.4.2/2.4.5 pattern.
	✓ Body elongated, ✓ A thin cartilaginous layer covering lower jaws, ✓ Scales hexagonal, ✓ A short pair of rostral barbells, ✓ Dorsal fins with 10-11 rays, ✓ Lateral line with 34 to 38 scales.
	✓ Elongated stream-lined body, short dorsal fin with anterior branched rays shorter than head, snout without lateral lobe, ✓ Inferior mouth, fringed and thick lipped lower jaw, ✓ 2 barbells, 12–15 rays in dorsal fin, Dorsal fin with 12-14 1/2 branched rays, ✓ lower profile of head conspicuously arched, 12-16 predorsal scales.
	✓ Mouth inferior, lips thin and continous, a small tubercule inside lower jaw above mandibular symphysis, young often with few irregular black spots on anterior scales of lateral line, Golden yellow above and on dorsal half of flank, ✓ Dorsal fin with 11 to 14 rays (branced rays 9 -10), one pair of minute maxillary barbells, not easily seen, Lateralline scales 37-40,
	✓ Small, inferior mouth surrounded by fleshy lips, ✓ The mouth is narrow and the lips are fringed, ✓ As the fry grows, the body and fins become blacker and finally at the fingerling stage *L. calbasu* becomes completely blackish-grey. ✓ Dorsal soft rays 16, anal soft rays 8,

	✓ Body elongated and dorsal profile is more convex than of ventral, ✓ Mouth blunt, narrow and subinferior, lips thick and fringed, ✓ Two very short pairs of barbels (maxillary and roastral), ✓ Pectoral fin as long as head, caudal fin deeply forked.
	✓ Body deep and compressed, single maxillary pair of barbels present, last simple dorsal ray moderately strong and smooth, ✓ Lateral line complete with 24-28 scales, scales from dorsal fin origin to lateral line 6 and those from lateral line to pelvic fin origin 4, ✓ Predorsal scales 11, circumpeduncular scales 14, pre-pelvic scales 11, pre-anal scales 19, ✓ Dorsal soft rays 11, Anal soft rays 8,
	✓ Body deep, barbels absent, dorsal fin spine osseous, moderately strong and serrated, lateral line incomplete. ✓ One of the hardiest of the barbs, undemanding and beautiful, ✓ Most impressively colored during the mating period, when the normally silvery male takes on a rich claret flush.
	✓ Max length : 4.6 cm TL male/unsexed, occurs in hill streams, ✓ Freshwater; benthopelagic, tropical, ✓ *Puntius fraseri* is endemic to Western Ghats of India, ✓ It inhabits hill streams and attains a total length of 4.6 cm.
	✓ Three diffuse black blotches on the body, first behind opercle, second below dorsal-fin origin, third above anal-fin origin, black spot at base and origins of dorsal, anal and pelvic fins, ✓ Dorsal soft rays 11, anal soft rays 8, ✓ Lateral line incomplete, with 3-4 pored scales, 21-22 + 1 scales in lateral series, ½4/1/2½ scales in transverse line on body, 8 predorsal scales.
	✓ Adult males tend to be noticeably slimmer than females and possess more intense colour pattern, the ventral fins are reddish in males, yellow in females, ✓ Inhabits sluggish rivers, streams, ponds and swamps which are often choked with algae or aquatic plants, ✓ Max length : 3.5 cm TL male/unsexed

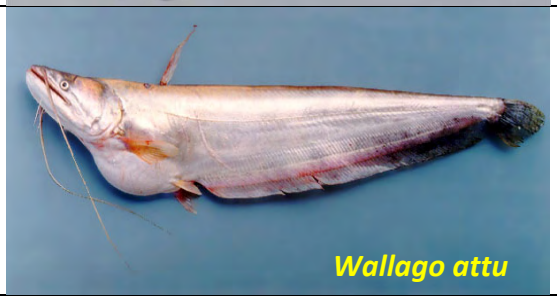
	✓ The body of olive barb is deep and moderately compressed; dorsal profile elevated. ✓ Eyes are large and situated in the anterior half of the head and snout is rounded, ✓ Maxillary pair longer than orbit, rostral pair shorter, ✓ Dorsal spines 3, Dorsal soft rays 8, ✓ Anal spines: 2; Anal soft rays: 5, ✓ Body oblong, head, small, barbels 2 pairs.
	✓ A dark spot present at the tip of the tail, another at tye base of the dorsal fin rays, ✓ Body is moderately compressed, mouth is small, terminal and upper jaw is slightly longer, ✓ Dorsal soft rays (total): 11-12; Anal soft rays: 8, ✓ Adults inhabit rivers, streams and ponds in plains and submontane regions,
	✓ Mouth is small and its position is termninal, barbells are absent, colour is silvery, ✓ Two black spot found on the lateral line which is incomplete, ✓ Depth of the body is less than one-third of the standard length, ✓ Maximum length is around 10.2 cm.
	✓ Maximum length records vary between 63 – 90 mm, ✓ Adult males are slightly smaller, slimmer, and display more intense colour pattern than females, ✓ Especially in the extent of yellow-golden pigmentation on the caudal peduncle.
	✓ Occurs in shallow and relatively deep areas of streams, both in still and relatively fast-flowing waters. ✓ Form shoals with 15-30 individuals, ✓ Dorsal soft rays (total): 10-11; Anal soft rays: 20 – 22, ✓ Body depth 2.8-3.6 times in SL; 17-21 branched anal rays conspicuous black stripe on posterior half of body.
	✓ Body is elongated and strongely compressed, ✓ Mouth is oblique, lower jaw with a well developed symphysial knob, ✓ Scales are very small, dorsal fin inserted well in advance anal fin, ✓ Lateral line is concave, Body colour is silvery.
	✓ Elongated body compressed laterally, ✓ Eyes situated on the anterior part of the head, ✓ Abdominal profile is cutting behind the base of the pectoral fin. ✓ Caudal deeply forked, lower lobe longer, ✓ Lateral line is complete and curves gently downwards.

	✓ Body laterally compressed and dorsal profile is more convex than ventral, ✓ No barbels, caudal fin deeply forked and lobes are pointed, ✓ Dark marking present in the dorsal and anal fins, ✓ Body colour light green on back and silvery on sides.
	✓ Anal fin distinctively striped, lateral line absent, ✓ Rostral barbels extend to anterior margin of orbit; maxillary barbels end at about middle of opercle, ✓ Vertebrae: 31 – 32, five uniformly, pigmented, horizontal stripes on the side of the body, all extending onto the end of caudal fin rays, ✓ Branched anal fin rays 10-12, vertebrae 31-32.
	✓ Max length : 10.0 cm TL male/unsexed, Inhabits rivers, canals, ponds, beels and inundated fields, ✓ Fish has high backed with a round belly and a forked caudal fin. ✓ The body is yellow in base colour, darker on the belly and in the fins, the top of the back is paler, with blue iridescence flanks with some yellow banding.
	✓ Body elongated and compressed laterally with pointed head, ✓ Lower jaw longer, moutrh small and two pairs of barbells of which maxillary pair is extremely long reaching middle of the body, ✓ Pectoral long and pointed, ✓ Lateral line incomplete.
	✓ Body elongate, oblong and compressed with small mouth, ✓ No barbels, ✓ Lateral line complete and descends very gradually, ✓ 21-34 scales on lateral line.
Order: Cypriniformes **Family: Cobitidae**	
	✓ Body elongated and laterally compressed, ✓ Dorsal and ventral profile almost straight and parallel, ✓ Caudal fin rounded with no lateral line, ✓ Pectoral fin with osseous spine in males, ✓ 3 pairs of barbels including 2 pairs of rostral and 1 pairs of maxillary barbels, ✓

Order: Siluriformes
Family: Bagridae

	✓ Maxillary barbels, in adults, extend posteriorly beyond the caudal fin base, but in young specimen, do not extend beyond the anal fin, ✓ Dorsal spine weak, often feebly serrated, A dark spot emphasized by a white or pale area along its ventral margin is just anterior to the first dorsal spine, ✓ Dorsal spines 1, Dorsal soft rays 7, Anal spines 0, Anal soft rays: 10 – 11, Body elongate and compressed, head conical, occipital process narrow.
	✓ Body elongated slightly compressed, head depressed, ✓ Dorsal spine long upto head, pectoral spine with 10-13 denticulations, ✓ Pectoral spine stronger than dorsal spine, ✓ 4-5 longitudinal bands along sides, ✓ Adipose short, upper lobe of caudal fin longer, ✓ Body colour yellow to brown with a dark spot on shoulder.
	✓ Maxillary barbels extending beyond pelvic fins, often to end of the anal fin, Dorsal spine weak, finely serrated on its inner edge. Adipose fin small, inserted much behind rayed dorsal, ✓ Color in life varies with age; generally delicate gray-silvery to shining golden, with several (about 5) pale blue or dark brown to deep black longitudinal on side, ✓ A narrow dusky spot often present on the shoulder. The fins glass, with dark tips, ✓ Dorsal spines 1, Dorsal soft rays 6-7, Anal spines 0, Anal soft rays 12 – 13, Vertebrae 31 – 37, Body elongate and slightly compressed.

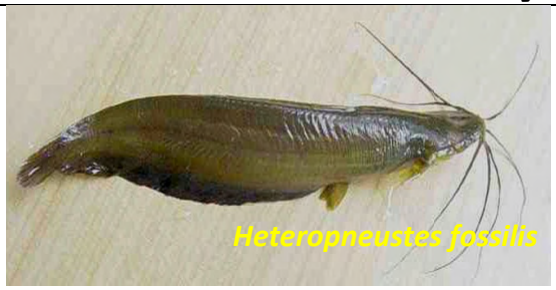
Order: Siluriformes
Family: Siluridae

	✓ Gap of mouth not extending beyond eye, ✓ Maxillary barbels lomg extend beyond anal fin base, ✓ Dorsal fin short with 4 rays, spine absent, ✓ Pelvic fin (7 to 9 rays) not reaching anal fin origin, ✓ Anal fin rays 60-75, ✓ One spot behind operculum.
	✓ Body elongate, strongly compressed, Mouth very deeply cleft, its corner reaching far behind eyes, ✓ Teeth in jaws set in wide bands, Barbels two pairs; maxillary barbels extending to anterior margin posterior of anal fin, mandibulary barbels to angle of mouth, Dorsal fin small, anal fin very long, ✓ Dorsal soft rays 5, Anal soft rays, 77 – 97, Head broad, snout depressed.

Order: Siluriformes
Family: Clariidae

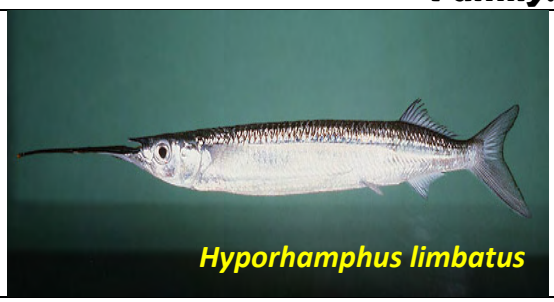
- ✓ Body compressed posteriorly. Upper jaw a little projecting. Spine of pectoral fins rough on its outer edge and serrated on its inner edge,
- ✓ Occipital process more or less triangular, its length about 2 time in its width, distance between dorsal and occipital process 4-5.5 times in distance from tip of snout to end of occipital process,
- ✓ Dorsal spines 0, Dorsal soft rays 60-76, Anal spines: 0; Anal soft rays: 47 – 58.

Order: Siluriformes
Family: Heteropneustidae



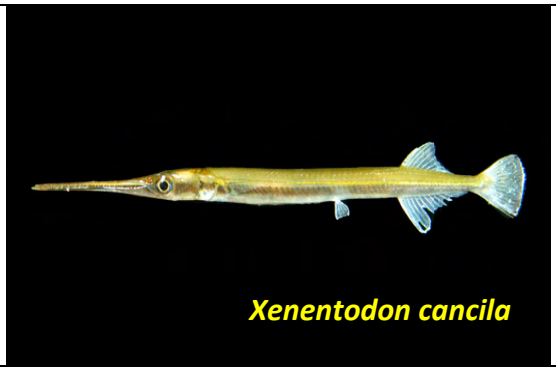
- ✓ Body elongated and compressed, depressed head covered with osseous plate on top and sides of head,
- ✓ Barbels four pairs in which maxillary pair extends to end of pectorals or to commencement of anal ands mandibular pair extend upto base of pelvic,
- ✓ Caudal fin rounded,
- ✓ A pair of accessory respiratory organ (air sacs) which extends backwards from the gill chamber.

Order: Beloniformes
Family: Hemiramphidae

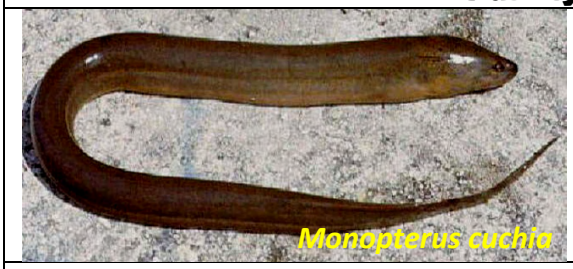


- ✓ Greatly prolonged, beak-like lower jaw, equal to, or longer than head length; upper jaw short, triangular and scaly, its width 0.6-0.8 times in its length.
- ✓ Preorbital distance 1.3-2.1 times in diameter of orbit and 0.75-1.2 times in length of upper jaw, Anal fin rays 13-16; caudal fin emarginate, not strongly forked,
- ✓ Dorsal spines 0; Dorsal soft rays 13-16; Anal spines: 0; Anal soft rays: 13 - 16.

Order: Beloniformes
Family: Belonidae



- ✓ Body very elongate and slightly compressed. Dorsal fin inserted usually anterior to a vertical through the origin of the anal fin,
- ✓ Green-silvery dorsally, grading to whitish below. A silvery band with a dark margin run along the side; a series of four or five blotches (absent in young specimens) on sides between the pectoral and anal fins. Dorsal and anal fins with dark edges,
- ✓ Dorsal spines 0; Dorsal soft rays 15-18; Anal spines: 0; Anal soft rays: 16 – 18.

Order: Cyprinodontiformes **Family: Aplocheilidae**	
 *Aplocheilus panchax*	✓ Mouth terminal, cleft of mouth wide, not extending to front border of orbit, ✓ Dorsal fin inserted behind posterior end of anal fin, dorsal with 8 soft rays, ✓ Anal fin square shaped with 15-16 rays, caudal fin rounded.
Order: Synbranchiformes **Family: Synbranchidae**	
 *Monopterus cuchia*	✓ A rudimentary dorsal fin originates a little anterior to vertical from anus, ✓ Presence of numerous spots all over tail, ✓ Eyes small, head not conspicuous, gill opening crescentic of which gill greatly reduced, ✓ Scales distinct and longitudinally arranged, ✓ Adults known to hibernate in mud during cold season.
Order: Perciformes **Family: Ambassidae**	
 *Chanda nama*	✓ Body is strongly compressed and laterally almost flat, ✓ Dorsal and ventral profile is equally convex, ✓ Lateral line partly distinct, partly absent, ✓ Scales are minute and rounded, Caudal fin forked, ✓ Caudal fin is black and orange, a small black spot found at the origin of the base of anal fin. ✓ Spines of first and second dorsal rays gradually decrease in height.
 *Pseudambassis baculis*	✓ Max length : 5.0 cm SL male/unsexed, ✓ Occurs in ponds, ditches, pools and rivers,
 *Pseudambassis lala*	✓ Small, deep-bodied, amber tinted fish. There are three vertical black bars on the flanks, and the dorsal and anal fins of the males have electric blue edges. ✓ Caudal fin rays 16-17 (upper lobe 8-9 lower lobe 8), Scales on body are very small (scales in lateral series ca. 6-70). Total vertebrae 24 (precaudal 10 caudal 14), Max length : 3.8 cm SL male/unsexed, ✓ Dorsal fin rays VII-I 12-13. Anal fin rays III, 13-14. Pectoral fin rays 10–11.

 *Pseudambassis ranga*	✓ Small, highly transparent species. Similar in shape to *Parambassis lala*, but instead of vertical bands on the flanks there is a single dark patch behind the eye. ✓ Max length : 8.0 cm TL male/unsexed, ✓ Males have pointed swimbladder, whereas females are rounded, Males also have blue edging on the dorsal and anal fins and have slightly deeper yellow colouration on the body than females, these colours are at their most vibrant when the fish are spawning.
Order: Perciformes **Family: Nandidae**	
 *Nandus nandus*	✓ An oval-shaped fish with an arched back and lateral compression, ✓ The first 12 rays of the long dorsal fin are spiny, while the rest are not. The caudal fin is fan-shaped and the mouth is deeply cleft, ✓ The body coloration change, although it is generally gray with irregular brown markings, ✓ The eye has two brown stripes passing through it: one running from the mouth to the origin of the dorsal fin, and the other running from the throat to the eye.
Order: Perciformes **Family: Badidae**	
 *Badis badis*	✓ The species has conspicuous dark blotch covering superficial part of cleithrum above pectoral fin base. ✓ Body depth 30.7-38.9% SL; interorbital width 6,5-8,3% SL; scales in lateral row 25-27; circumpeduncular scales 19-20; pectoral rays usually 12. ✓ Has a series of prominent dark blotches along dorsal fin base and/or a series of dark blotches along middle of dorsal fin; and has indistinct bars on side. Has a distal extrascapular, ✓ Dorsal spines 15 - 17; Dorsal soft rays 7-10; Anal soft rays: 6 - 8; Vertebrae: 26 - 28.
Order: Mugiliformes **Family: Mugilidae**	
 *Rhinomugil corsula*	✓ Body is sub-cylindrical in anterior region and moderately compressed in posterior, ✓ Dorsal profile is nearly straight, ✓ Head is flat in above and compressed at sides ✓ Dorsal spines (total): 4; Dorsal soft rays (total): 1-8; Anal soft rays: 3 – 9.

Order: Perciformes
Family: Gobiidae

- ✓ Head flattened, lower jaw projecting; body pale without longitudinal lines,
- ✓ The body is brownish yellow with 5 to 6 dark and rounded spots on its sides.
- ✓ Dorsal fins are light with brownish spots. Pelvic fins are grey. Pectorals and caudal are grey and often hyaline,
- ✓ Dorsal spines 7; Dorsal soft rays 8-9; Anal spines: 1; Anal soft rays: 8 - 9.

Order: Perciformes
Family: Anabantidae

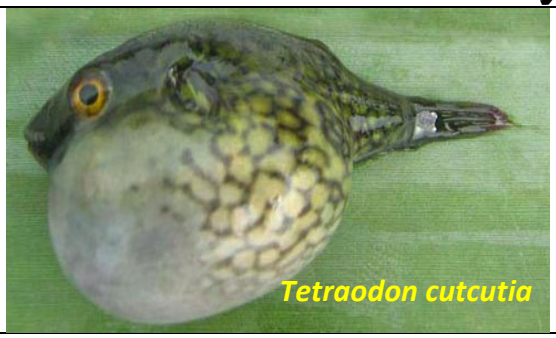
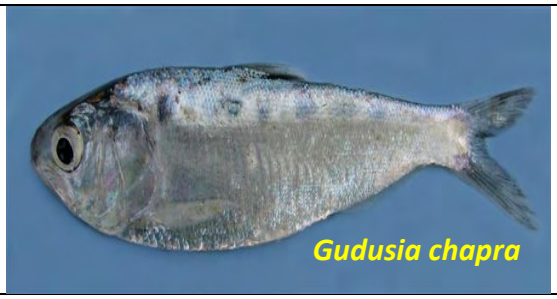
- ✓ Color in life dark to pale greenish, very pale below, back dusky to olive; head with longitudinal stripes ventrally; posterior margin of opercle with a dark spot; iris golden reddish.
- ✓ Body form variable, affected by age and amount of food consumed. Scaled head with 4-5 rows between eye and rear margin of preoperculum. Scales large and regularly arranged, ciliate,
- ✓ Dorsal spines 16 - 20; Dorsal soft rays 7-10; Anal spines: 9-11; Anal soft rays: 8 - 11.

- ✓ Body elongate and strongly compressed.
- ✓ Mouth small, slightly protrusible; upper lip papillose, especially in old males. Preorbital serrate in young specimen. Color greenish with oblique orange or bluish bars descending downwards and backwards from the back to the anal fin,
- ✓ Dorsal spines 15 - 17; Dorsal soft rays 9-14; Anal spines: 15-18; Anal soft rays: 14 - 19; Vertebrae: 27.

- ✓ The wild form of Dwarf Gouramis have alternating blue and red/orange vertical stripes covering the body, with the stripes extending into the fins,
- ✓ The females are generally a dull silvery blue colour,
- ✓ Dwarf Gouramis are a relatively small, stumpy Gourami with long ventral fins that look like feelers,
- ✓ Inhabits slow moving streams, rivulets and lakes with plenty of vegetation

- ✓ A pretty and small Gourami, a similar shape to the common Dwarf Gourami, with long ventral 'feelers',
- ✓ The body is coloured a deep ochre colour, can be more of a pale yellow, whereas the dorsal, caudal and anal fins are a deep orange,

Order: Perciformes Family: Channidae	
 *Channa marulius*	✓ Max length : 183 cm TL male/unsexed; common length : 46.0 cm TL male/unsexed; max. published weight: 30.0 kg, ✓ The Cobra Snakehead has dark eyes, a dipped snakelike head with jutting bottom jaw and is brown/gold-tinted to pale gray to dark brown with large black blotches. It has a distinctive marking which is the black spot rimmed with orange near the base of the tail fin, known as an eyespot or ocellus.
 *Channa gachua*	✓ White dorsal, anal and caudal margins; 3-3+1/2 scales between the lateral line and the base of the anterior dorsal rays; relatively small size, ✓ Max length : 20.0 cm SL male/unsexed; common length : 5.0 cm SL male/unsexed, ✓ In most populations males develop slightly more-extended dorsal and anal fins than females, and sometimes more intense colouration.
 *Channa panctatus*	✓ Max length : 31.0 cm TL male/unsexed; common length : 15.0 cm TL male/unsexed, ✓ Eyes are comparatively smaller in size and located at anterior of the head, ✓ Two pairs of nostrils located anterior superior angle of the eye, ✓ Lower jaw is slightly protruding, ✓ Body colour brown on back not spotted or striated.
 *Channa striatus*	✓ Body sub-cylindrical; head depressed; caudal fin rounded. ✓ The dorsal surface and sides is dark and mottled with a combination of black and ochre, and white on the belly; a large head reminiscent of a snake's head; deeply-gaping, fully toothed mouth; very large scales, ✓ Dorsal spines (total): 0; Dorsal soft rays (total): 38-43; Anal spines: 0; Anal soft rays: 23 - 27.
Order: Synbranchiformes **Family: Mastacembelidae**	
 *Macrognathus aral*	✓ Its basic colour is olive to light brown, and while there is certainly a single band running horizontally along each flank, there is also a dark band along the dorsal surface. ✓ These bands are rather irregularly coloured, often being speckled or being darker on the edges and lighter in the centre. The dorsal fin bears a few (typically four) eyespots that are light brown around the edge and dark brown in the middle.

 *Macrognathus pancalus*	✓ Macrognathus pancalus, the barred spiny eel or Indian spiny eel, is a small fish from southern Asia. ✓ It usually is found in slow and shallow rivers. Males are more slender and often smaller than the females. ✓ Max length : 18.0 cm TL male/unsexed, ✓ Inhabits slow and shallow waters of rivers of plains and estuaries; never available above an altitude of 366 m.
 *Mastacembelus armatus*	✓ Both are light brown with dark brown markings are concentrated on the top half of the body, leaving most of the belly bare, ✓ Dorsal spines 33 - 40; Dorsal soft rays 67-82; Anal soft rays: 67 - 83; Vertebrae: 87 – 98, ✓ Body dull brown with 1-3 darker, longitudinal zigzag lines, more or less connected to form a reticulated pattern, more or less distinct and restricted to the dorsal two thirds of the body.
Order: Tetraodontiformes **Family: Tetraodontidae**	
 *Tetraodon cutcutia*	✓ Max length : 15.0 cm TL male/unsexed; common length : 10.0 cm TL male/unsexed, ✓ Broad head and back is tapering abruptly to tail, ✓ Mouth opening is a little inferior with two large teeths on each jaw, ✓ Gill opening is reduced and restricted to pectoral base, ✓ Eyes are large and situated slightly behind middle of head.
Order: Clupeiformes **Family: Clupeidae**	
 *Gudusia chapra*	✓ A single triangular pectoral axillary scale; depressed tip of dorsal fin to behind vertical from anal fin origin. Hind margin of scales smooth, ✓ Dark blotch behind gill opening, often followed by a series of spots along flank. Gill rakers fine and numerous, increasing with size of fish, ✓ Dorsal spines 0; Anal spines: 0. Body fairly deep; 26 to 29 scutes along belly,
Order: Osteoglossiformes **Family: Notopteridae**	
 *Notopterus notopterus*	✓ Distinguishable by its plain brown adult coloration and the convex or only slightly concave dorsal head profile, ✓ Juveniles (<5 cm SL) with dark bars on the whole body, preopercular scale rows 6-8, silvery-white with numerous fine grey spots on body and head, ✓ Dorsal soft rays (total): 7-9; Anal soft rays: 97 - 111.

Order: Anguilliformes Family: Anguillidae	
	✓ Dorsal soft rays (total): 250-305; Anal soft rays: 220 - 250; Vertebrae: 106 - 112. Body elongate, head conical, flattened dorsally, ✓ Mouth terminal, lips prominent, narrow bands of teeth on jaws, broad band on vomer.

COMMON GEARS FOR FISHING IN WETLANDS

The gears used for fishing in wetlands is as diverse as the diversity of fishes and ecotones of wetlands known forApplication of gears and crafts in fishery is a result of experiences gained over a long period of time. Every water body has its unique pattern of gears. Since the pattern and regulation of fishing has a great bearing on the fish population dynamics, it is very important to study the nature of gears commonly used that may lead to selective fishing, thus minimizing stress on a particular size group of fishes (Srivastava and Srivastava 2011). Some of the common gears used for fishing in wetlands are:

Fishing Gears

Cast net (Chhabi Jal)
Cast net or Chhabi Jal is the main fishing gear operated in inland open waters. Cast net is a small bell - shaped net with weights on the periphery and having a string. It is operated by throwing the net in a fashion that forms a circle while falling on water for trapping the fishes in a water body (Figure.1). Cast net is operated in rivers, beels and ponds throughout the year. Fishes like Indian Major Carp, *Labeo bata*, *Hypophthalmichthys molitrix*, *Ctenopharyngodon idella*, *Puntius* spp., *Mystus* sp, and others are caught.

Gill net (Phansi Jal)
Gill net locally called Phansi jal is commonly used to catch fishes by gilling. Fishes which try to pass through it get gilled. There is great variation in mesh size of the gill net depending upon target species and varies from 0.6-7.5 cm in wetland ecosystem. Generally, rectangular in shape and is provided with a head rope of polypropylene carrying floats and a foot rope with or without sinkers. Besides cat fishes such as *Mystus* sp., *Heteropneustes fossilis*, *Clarias batrachus*, *Wallago attu*, fishes like *Gudisia chapra Channa* spp., *Anabas* sp, *Puntius* spp, *Mastacembelus* sp., and *Labeo rohita* and so on are caught. It is also a major fishing gear operated in rivers and beels throughout the year.

Lift net (Sitki Jal)
The Lift net or Sitki jal is a square net. The four corners of which are tied to the tip of two crossed flexible bamboos. A bamboo is attached to the point of crossing and the whole arrangement may or may not have a rope. Lift net is usually operated in the monsoon months. The major catch composition includes *Puntius* spp., *Amblypharyngodon mola* and *Barilius* sp.

Drag net (Masari jal and Bed jal)
Drag net locally called as Masari jal and Bed jal. This net, which is widely used, is structurally rectangular in shape and has a head rope carrying floats and a foot rope with or without sinkers. The net is usually operated in clear zone of the lake throughout the year. The mesh size ranges between 3-

8mm. Most of the pond fishes such as Indian Major Carp, Exotic Carp, *Labeo bata*, *Puntius* spp., *Mystus* sp, *Notopterus* sp and air- breathing fishes are caught by drag net.

Cloth net (Tana Jal)
The cloth net is a fine meshed mosquito net. It is locally called Tana jal. During fishing by cloth net, two persons hold the net at opposite ends and lift it from the water when sufficient number of fishes are trapped. Cloth net is implemented throughout the year except monsoon months in ponds. The fishes are usually caught by cloth net are *Puntius* spp., *Amblypharyngodon mola* and *Esomus denricus*.

Push net (Thela Jal)
Push net or Thela jal is a very common net used by local fishermen to catch fishes in the lentic and lotic water bodies (Figure.1). It is made up of a triangular bamboo frame fitted with a mosquito netting cloth. Fishermen operate it by pushing it in the water body and are used throughout the year. Fishes like *Trichogaster sp*., spawn and fry of Murrels, *Puntius* spp., *Amblypharyngodon mola* and *Esomus denricus* are usually caught by the Push net.

Triangular Brush Park
Local name: Dhara jakhe (Assam)
This triangular brush trap, made by weaving finely split bamboo strips, have the front part open. Tree/ bamboo branches are put inside the trap and it is partially submerged in shallow areas of small rivers. The fish taking shelter inside the trap are caught after 7-10 days by lifting the trap and removing the branches (Figure 2).

Conical brush trap
Local name: Dalangi, hukuma (Assam), Ruh ship (Meghalaya)
It is a medium size brush trap of conical shape. This is set in river or beels usually after the southwest monsoon (August-Navember) and is fastened to stakes driven into the bank with ropes. The inside of the trap is loosely filled with branches of bamboo/ tress to provide shelter to fishes. Animal offals or mustard oil cake is kept in this tube to attract fishes like spiny eels, walking catfish, etc (Figure 2).

Dome-shaped Trap (Vertical)
Local name: Ubhati, runga (Assam)
This is a dome-shaped trap used in marginal areas of floodplain wetlands of Assam. It varies widely in size (0.2-0.3m in diameter and 0.3-0.5 m in height). There is a small rectangular tunnel mouth at one side leading to a second funnel-shaped tunnel towards the dome. The trap is set vertically and baits of snail meat is usually kept inside the trap to attract fishes especially *Mystus* spp (Figure 2).

Cube-shaped Box Trap with Single/Double Inlet
Local name: *Boldha, kholha, Tai jep* (Manipur)
This trap made of wooden strips is rectangular in shape having a vertical inlet at the narrow side. The inlet structure is 'V' shaped and the sticks from either end touch together at the middle resembling, spines thereby preventing the trapped fish from moving out. These are variable in size (0.4-0.6 m long, 0.2-0.3 m wide and 0.5-0.7 in height). It is operated either for or against water current to catch miscellaneous fishes in shallow areas. Baits of snail meat are sometimes kept inside these traps to attract the fishes, especially *Mystus* spp (Figure 2).

Conical Falling Net
Local name: Chak jal (Assam)
It is a conical net fitted with a conical (pentagonal) bamboo frame. This falling gear is operated in shallow, macrophyte infested areas of beels from boats. After plunging the net, the rope of the conical net tied on to the top of the cone is untied from the frame. The fisher then steps over the net and catches the fishes by hand (Figure 1).

Fishing Crafts

Banana Raft
Local names: Kolar bhel (Assam), Kola gachher bheura (Tripura), Laphu paou (Manipur)

It is the cheapest and easily made craft used by poor fishers. Banana trees are cultivated by most households of the Northeast. For constructing this raft, 4-5 matured banana tree trunks are joined together (keeping all the bigger ends of the trunks towards the stem side) by hammering 2-3 split bamboo strips (khila) through the trunk along the transverse axis. The front of the raft is made pointed by cutting the sides to reduce resistance of water while moving through it. Commonly used banana rafts are 1.5-2.5m long and 1.01.2m wide (Figure.1). These rafts have the advantage of never sinking in water. However, these are quite heavy to push through water (done with the help of bamboo poles) and have a very short life span (3-4 weeks). These are used for operating cast net, gill nets, and line fishing mainly in shallow, still waters (Bhattacharjya et al. 2004).

Bamboo Raft
Local name: Bahor bhur (Assam), Banser veura (Tripura), Paou (Manipur)

It is another cheap and easily made craft used by poor fishers since bamboo is abundantly available in the region. About 20-50 light weight variety bamboos (e.g. Jati) are tied together keeping all the bigger ends of the trunks towards the stern side) with coir/jute ropes for constructing this raft. These rafts are usually 0-12m long and their width is variable (1.5-5.0 m depending on the water current and number of bamboos use). These rafts are also heavy to push through water (done with the help of bamboo poles) and have a moderate life span (1-2 years). They are usually used in sluggish rivers and floodplain lakes.

Dug-out Canoe
Local name: Tulunga nao, Donga (Assam), Dingi nouka (Tripura), Lukai hee (Manipur)

These are small wooden canoes dug-out from a single log of the tree. Naturally, there is a limitation on the overall length (3-4 m) and width/ depth (0.5-0.6 m) of these boats (Figure.1). Trunks of palm trees are also occasionally used to make smaller canoes. Construction of these canoes requires good craftsmanship and suitable (long and straight) wooden logs. Because of their small size, these canoes can accommodate only 1 or 2 fishers. Also, because of their narrow width, there is a lot of rolling movements (hence the name tulunga nao) requiring skills to maneuver them. Consequently, these are usually used in shallow floodplain lakes and other wetlands to carry and set fish traps, gill nets and lines. These canoes have the advantage of being leak proof (since these are curved out of a single tree log and have no joints). However, they are becoming rare because of spiraling suitable tree logs (e.g. mango) and labour. These are constructed from logs of certain trees like *Artocarpus chaplasa*, *Cedrella tuna* and *Plioeba* spp (Bhattacharjya et al. 2004).

Figure 4. Common crafts and gears used in open water ecosystem

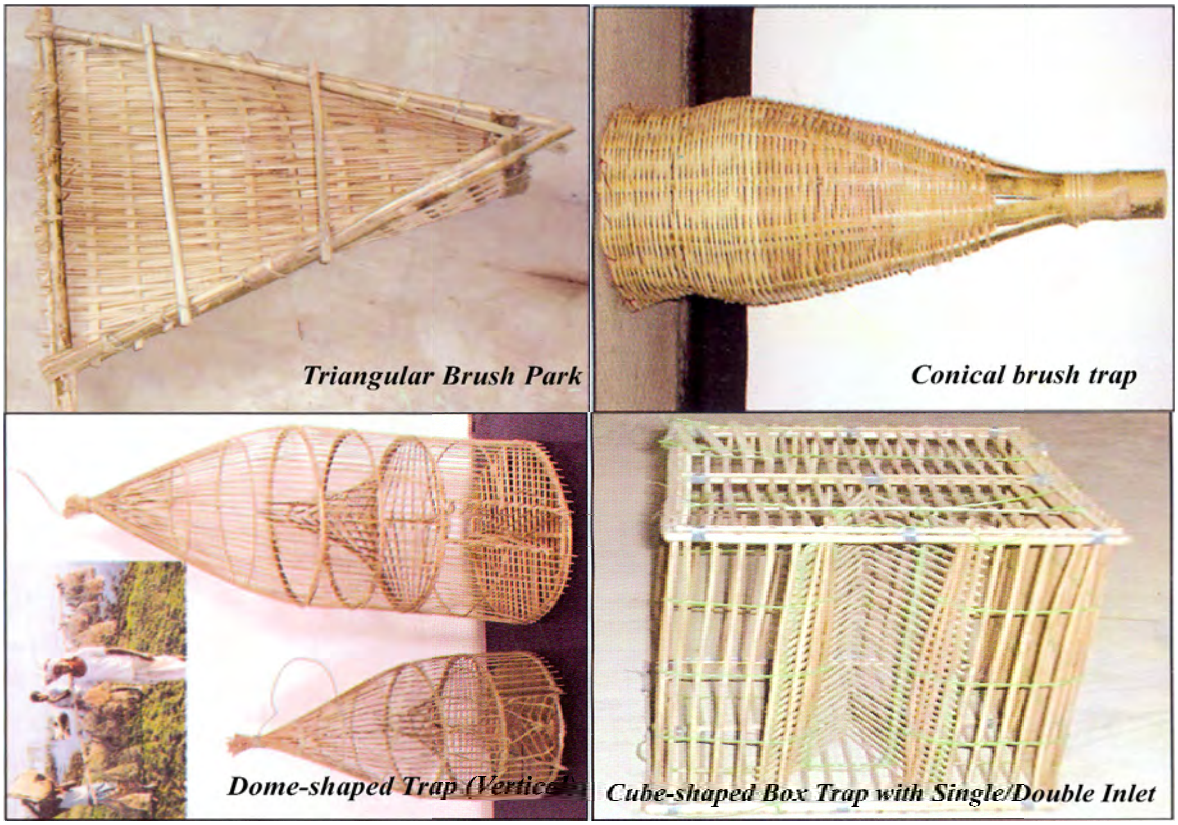


Figure 5. Common traps used in open water ecosystem

ECOSYSTEM SERVICES OF FISHES

Fish constitute one of the major protein sources for humans around the world. There are to date some 25 000 different known fish species of which 15 000 are marine and nearly 10 000 are freshwater (Nelson 2006). Fish populations provide ecosystem services for human societies, and the relations between these services and functioning ecosystems in different regions of the world. Small-scale freshwater ecosystems, on the other hand, are better understood in terms of influences of fish on ecosystem structure and function (Carpenter et al. 1985).

Ecosystem services can be broadly classified into two major categories: fundamental and demand-derived ecosystem services (Table 2). Fundamental ecosystem services are essential for ecosystem function and resilience, such as nutrient cycling. These are ultimately a prerequisite for human existence, irrespective of whether humans are aware of it or not. Such services are often not linked to any specific economic market value. The 'demand-derived ecosystem services', such as recreational values, are formed by human values and demands, and not necessarily fundamental for the survival of human societies. Nevertheless, all demand-derived ecosystem services ultimately depend on natural systems and the fundamental ecosystem services provided by fish, and are not replaceable by technological innovations (Holmlund and Hammer 1999).

Table 2 Major fundamental and demand-derived ecosystem services generated by fish populations

Fundamental ecosystem services	
Regulating services	**Linking services**
Regulation of food web dynamics	Linkage within aquatic ecosystems
Recycling of nutrients	Linkage between aquatic and terrestrial ecosystems
Regulation of ecosystem resilience	Transport of nutrients, carbon and minerals
Redistribution of bottom substrates	Transport of energy
Regulation of carbon fluxes from water to atmosphere	Acting as ecological memory
Maintenance of sediment processes	
Maintenance of genetic, species, ecosystem biodiversity	
Demand-derived ecosystem services	
Cultural services	**Information services**
Production of food	Assessment of ecosystem stress
Aquaculture production	Assessment of ecosystem resilience
Production of medicine	Revealing evolutionary tracks
Control of hazardous diseases	Provision of historical information
Control of algae and macrophytes	Provision of scientific and educational information
Reduction of waste	
Supply of aesthetic values	
Supply of recreational activities	

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