CENTRAL POLLUTION CONTROL BOARD
PariveshBhawan, East Arjun Nagar, Delhi-110032
BIO-SCIENCE LABORATORY

Field Protocol for Bio- Monitoring

SAMPLING EVENT DETAILS	:	
Water body	:	
Location	:	
Data of sampling	:	
Staring time of sampling	:	
Ending time of sampling	:	
Sampling team	:	

LOCATION DETAILS		1	2	3	4	5	6	7	8	9	10	d
Avg. Depth (m) or feet	:											
(Approx depth can be measured from Height of cattles wading, from bathing activities, length of Iron rope of artificial substratumin water body or any other measuring device)	:											
Approx. width (m or feet)	:											
Approx. main stream flow (m/s)	:											

Description of water	
Pool	
Slack	
Riffle	
Run	
:Depositing	
Eroding	
Turbulent	
Canalized	

Substrate composition of Natural orManmade water body/wetland :

>256 mm	Boulders	0%
255-64 mm	Cobbles	0 %
63-16 mm	Pebbles	0%
15-2 mm	Gravel	0%
0.0625 mm	Sand	0%
0.002 mm	Silt	0%
<0.002 mm	Clay	0%
	Detritus	0 %
	Macrophytic vegetation	0%
	Artificial substratum	0%

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Human influences : Melon farming

Cattle wading

Dredging

Sand recovery

Other

Wet land plants

Macrophytecover/ Name :

Birds /wild life habitation /Name :

Surrounding land use : Urban

Arable

Grazing

Forest

Other

Sun's intensity : Nil

Moderate

Heavy

Approx altitude (m) :

Discharges :

Confluences :

Make a detailed drawing or photograph of the local situation, and indicate sampling station and other peculiarities:

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Check list for Bio- Monitoring

1. Sieve with 0.6 mm mesh size
2. Hand net
3. Shovel
4. Scrapper
5. Depth measurement device- Folding stick etc.
6. Plastic ball, measuring tape & stop watch for flow measurement
7. Gum boots and hand gloves
8. pH strips, DO bottles & reagents
9. Thermometer
10. White enamel trays
11. Small plastic bucket & rope
12. Wide mouth bottle
13. Forceps, needles & convex hand lens
14. Formalin (4%) or Alcohol (70%)
15. Stickers & marking pen
16. Artificial substratum & its accessories for lined canals etc.
17. First aid box, soap disinfectant & towel
18. Field protocols
19. Camera for site photograph if required, caps etc
20. One big box/crate to accommodate artificial substratum for bio-monitoring of benthic macro-invertebrate.
21. Water proof file /bag for placing the field protocols
22. Life Jacket

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Check List for Sample Collection:

SAMPLETYPE	BOTTLE	VOLUME	PRESERVATION	TICKMARKTHE SAMPLE COLLECTED FROM THE SITE

Water Samples for Chemical Analysis

Physico-chemical Composition	PE Carboy	5 L	Cooled in ice	
Ammonia	G NM	1 L	2 ml $H_2 SO_4$	
Heavy metals	PENM	250ml	1 ml HNO_3	
Mercury	GNM	250ml	1 ml HNO_3 +5ml $K_2 Cr_2 O_7$	
Pesticides	GNM(brown)	1 L	-	
PAH+ PCB + etc.	GNM(brown)	1 L	-	
Oil and grease	GNM(brown)	1 L	Cooled in ice	
Phenol	GNM	1 L	Cooled in ice	
Chlorophyll	GNM(brown)	1 L	-	
	GNM GS(Sterilized)	300 ml	Cooled in ice	

Water samples for Bacteriological Analysis

Total Coliform and Fecal Coliform	Sterilized Glass Bottle	125ml	Cooled in ice	

Sediment Samples for Chemical Analysis

Heavy metals	PE WM	1 L	Cooled in ice	
Pesticides + PAH + PCB + etc.	G WM	1 L	Cooled in ice	

Biological Samples for Chemical Analysis (Fish, Mussels, Water Hyacinth,etc)

Heavy metals	PE bag		Deep frozen	
Pesticides + PCB + etc.	G WM	1 L	Deep frozen	

Biological Samples for Bio- Assessment

Benthic Macro-Invertebrates	PE WM	0.1-0.5L	Alcohol 70%or Formalin (4%)	

Others

Effluent toxicity	PE Carboy	25 L	None	
Up-stream dilution water	PE Carboy	25 L	None	

G-Glass, GS – Glass stoppered, PE- Polythene, NM- Narrow Mouth, WM –wideMonth

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FLELD MEASUREMENTS:

Reference Thermometer Code:

Time	Date	Water temperature (^{0}C)			Air temperature (^{0}C)			DO Titration(mg/1)	pH strip
		Temp Observed	Correction factor	Actual Temp	Temp Observed	Correction factor	Actual Tamp		
AM		^{0}C		^{0}C	^{0}C		^{0}C	mg/l	

SAMPLING OF BIOLOGICAL ORGANISMS

PROCEDURE: - Different procedures can be employed for sampling of biological parameters. Sampling should be conducted during availability of ample amount or sunlight in the field. Find out the nature of river bed and select the procedure. Always approach the sampling area starting from downstream to upstream. Avoid bio- monitoring at places of acute air quality problems.

I. Stoney River Bed:

- Pick up stones randomly from the fast flowing shallow stream and remove the organisms by brush or soft forceps pins into a white tray.
- Place the sampling net firmly on to the stream bed.
- Brush off the large stones or rocks lying under water and placed adjacent to the mouth of net for collecting animals.

II.Smaller Stones and sandy Bed:

- Place the net or sieve firmly on the stream bed against the flow. Stand before the net or sieve and kick up the stream bed by foot and collect the animals into the net or sieve. Wash the animals into white tray.

III.**Mud and Silty bed:**

- Pick up 5grab samples of the river bed by the shovel. Wash the sample in the sieve by river water pick up the animals by hand or brush or soft forcep pins into white tray.

IV. **Water plants / floating lands:**

- Uproot the water plants present near the sampling area. Wash and collect the animals either directly into the net or into white tray.
- Collect the benthic macro –invertebrates from floating land by scrubbing the sieve or net under or sides of the floating land.
- Identify the indicator animals belonging to various taxa from the given identification char and the characters, mote sown the abundance of each animals identified. Compare the results from the water quality Evaluation system of BWQC and define the water quality class to the investigated water body.

SAPROBIC [BIOLOGICAL MONITORING WORKIING PARTY (BMWP) SCORE:

Ample care should be taken to ensure that all indicator families of Benthic Macro- invertebrates, which are present, are actually encountered. This can be accomplished by sub- sampling al different (micro) habitats in a sizeable stretch of the river /water body. The monthly inventory fieldwork can be restricted to a biologically mature period of the year (October-May), excluding monsoonand periods, Use identification key at page 12 for preliminary identification of Benthic Macro –invertebrates Taxa.

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This method involves a quantitative inventory of the presence of macro –invertebrate benthic fauna up to family/genus level of taxonomic precision. All possible families having saprobic indicator value are classified on a score scale of 1to 10 according to their preference for saprobic water quality. The families which are most sensitive to pollution are on the top of the list (Table 1) and are getting a score of 10 while the most pollution tolerant families are getting a scope of 1 and 2. The other intermediately sensitive families are placed in between the scoping scale of 10 to1.

ENTER DIFFERENT SPECIES WITHIN ONE FAMILY SEPARATEY, AND INDICATE ABUNDANCYAS:

Abundance scale**: A =single (one individual0**
B =scarce (2-10individuals)
C =common (10-50 individuals)
D =abundant (50-100 individuals)
E = excessive (more than 100 individuals or only one species)

TABLE-1

TAXONOMICAL GROUP	TAXONOMICAL FAMILIES	MARK ENCOUNTERED FAMILIES AND IF POSSIBLE GENUS/ SPECEIES WITHHIN FAMILIES. ALSO MARK ABUNDANCY AS 1A,1B,1C,1D,1E,	TOAL FAMILIES/GENUS/SPECIES ENCOUNTERED	BMWP SCORE	MULTI-PLIED SCORE
Ephemeroptera	Siphonuridae				
	Heptageniidae				
	Leptophlebiidae				
	Ephemerelidae				
	Pothaminthidae				
	Ephemeridae				
	Prosopistomatidae				
Plecoptera	Taeniopterygidae				
	Leuctridae				
	Capniidae				
	Perlodidae				
	Perlidae				
Hemiptera	Aphelocheiridae				
Trichoptera	Leptoceridae				
	Goeridae				
	Lepidostomatidae				
	Brachycentridae				
	Sericostomatidae				
TOTAL FAMILIES ENCOUNTERED & TOTAL MULTIPLIED SCORE				**X 10**	

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TAXONOMICAL GROUP	TAXONOMICAL FAMILIES	MARK ENCOUNTERED FAMILIES AND IF POSSIBLE GENUS/ SPECEIES WITHHIN FAMILIES. ALSO MARK ABUNDANCY AS 1A,1B,1C,1D,1E,	TOAL FAMILIES GENUS/SPECIES ENCOUNTERED	BMWP SCORE	MULTI-PLIED SCORE
Odonata	Euphaeidae				
	Protoneuridae				
	Plathycnemididae				
	Gomphidea				
	Cordulegasteridae				
	Aeschnidae				
	Corduliidae				
	Libellulidea				
Trichoptera	Psychomyiidae				
	Philopotamidae				
TOTAL FAMILIES ENCOUNTERED & TOTAL MUTIPLIED SCROE				**X8**	
Ephemeroptera	Caeanidae				
Plecoptera	Nemouridae				
Trichoptera	Rhyacophilidae				
	Pclycentropodidae				
	Limnephilideae				
TOTAL FAMILIES ENCOUNTERED & TOTAL MULTIPLIED SCORE				**X7**	
Mollusca	Neritidae				
	Viviparidae				
	Thiaridae				
	Pleuroceridae				
	Bithynidae				
	Amblemidae				
	Ancylidae				
	Unionidae				
Trichoptera	Hydroptilidae				
Crustacean	Atydae				
	Gammaridae				
	Paleamonidae				
	Potamidae				
	Parathelphusidae				
Polychaeta	Nereidae				
	Naphthyidae				
Odonata	Agriidae				
	Coenagrionidae				
TOTAL FAMILIES ENCOUNTERED & TOTAL MULTIPLIED SCORE				**X6**	

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TAXONOMICAL GROUP	TAXONOMICAL FAMILIES	MARK ENCOUNTERED FAMILIES AND IF POSSIBLE GENUS/SPECEIES WITHHIN FAMILIES. ALSO MARK ABUNDANCY AS 1A,1B,1C,1D,1E,	TOAL FAMILIES GENUS/SPECIES ENCOUNTERED	BMWP SCORE	MULTI-PLIED SCORE
Hemiptera	Mesovelidae				
	Hydrometridae				
	Gerridae				
	Nepidae				
	Ranatrinidae				
	Naucoridae				
	Notonectidae				
	Pieidae				
	Veiiidae				
	Hebridae				
	Beiastomatidae				
	Corixidae				
Coleoptera	Heteroceridae				
	Hygrobidae				
	Dytiscidae				
	Gyrinidae				
	Hydrophilidae				
	Dryopidae				
	Eiminthidae				
	Noteridae				
	Psephenidae				
Trichoptera	Hydropsychidae				
Diptera	Tipuiidae				
	Tabanidae				
	Cuiicidae				
	Blepharoceridae				
	Simulidae				
Planaria	Planariidae				
	Dendrocoelidae				
TOTAL FAMILIES ENCOUNTERED & TOTAL MULTIPLIED SCORE				**X5**	
Ephemeroptera	Baetidae				
Megaloptera	Sialidae				
Hirudinea	Piscicolidae				
TOTAL FAMILIES ENCOUNTERED & TOTAL MULTIPLIED SCORE				**X4**	

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TAXONOMICAL GROUP	TAXONOMICAL FAMILIES	MARK ENCOUNTERED FAMILIES AND IF POSSIBLE GENUS/SPECEIES WITHHIN FAMILIES. ALSO MARK ABUNDANCY AS 1A,1B,1C,1D,1E,	TOAL FAMILIES/GENUS/SPECIES ENCOUNTERED	BMWP SCORE	MULTI-PLIED SCORE
Mollusca	Lymnaeidae				
	Physidae				
	Planorbidae				
	Sphaeridae				
	Corbiculidae				
Hirudinea	Glossiphonidae				
	Hirudidae				
	Erpobdellidae				
Planaria	Dugesiidae				
Crustacea	Asellidae				
	Cirolanidae				
TOTAL FAMILIES ENCOUNTERED & TOTAL MULTIPLIED SCORE				**X3**	
Dipteral	Syrphidae				
	Chironomidae				
	Ephydridae				
TOTAL FAMILIES ENCOUNTERED & TOTAL MULTIPLIED SCORE				**X2**	
Oligochaeta	Tubifiscidae				
	Naididae				
	Octochaetidae				
	Lumbricidae				
TOTAL FAMILIES ENCOUNTERED & TOTAL MULTIPLIED SCORE				**X1**	
GRAND TOTAL FAMILIES ENCOUNTERED & GRAND TOTAL MULTIPLIED SCORE					

Saprobic score:**GRAND TOTAL MULTIPLIED SCORE**

GRAND TOTAL NUMBER OF FAMILIES ENCOUNTERED

SAPROBIC SCORE:

REMARKS:

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DIVERSITY SCORE (SEQUENTIAL COMPARISION):

The evaluation of the benthic fauna diversity leval can easily be done utilizing: the same animals collected for estimating the saprobicscore. Take photograph of the living animals in the field for evidence.

Sincethe method only involves a pair –wise comparison of sequentially encountered individuals and the differences of twospecimens can easily be observed up to the genus/species level, no taxonomic skill is required.

First observed animals is always different and scored as 1 run. When the next observed animal is different from the last, a new run starts. The encounter of an individual which cannot be discerned for the last does not increment the number of runs. Size differences only do NOT change the run.

SAME RUN IS 0(organism is the same as the previous)
NEXT RUN IS 1 (organism is different from the previous)

When a row is full, continue on next row. Enter the number of runs over all rows (sum of1's)

No	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	Total Runs	Total Org.	Diversity Score
1	1																15	
2																	30	
3																	45	
4																	60	
5																	75	
6																	78	
7																	105	
8																	120	
9																	135	
10																	150	
11																	165	
12																	180	
13																	195	
14																	210	
15																	225	
16																	240	
17																	255	
18																	270	
19																	285	
20																	300	

DIVERSITY SCORE: $\frac{\text{Number of Run}}{\text{Number of Organisms}}$

DIVERSITY SCORE:

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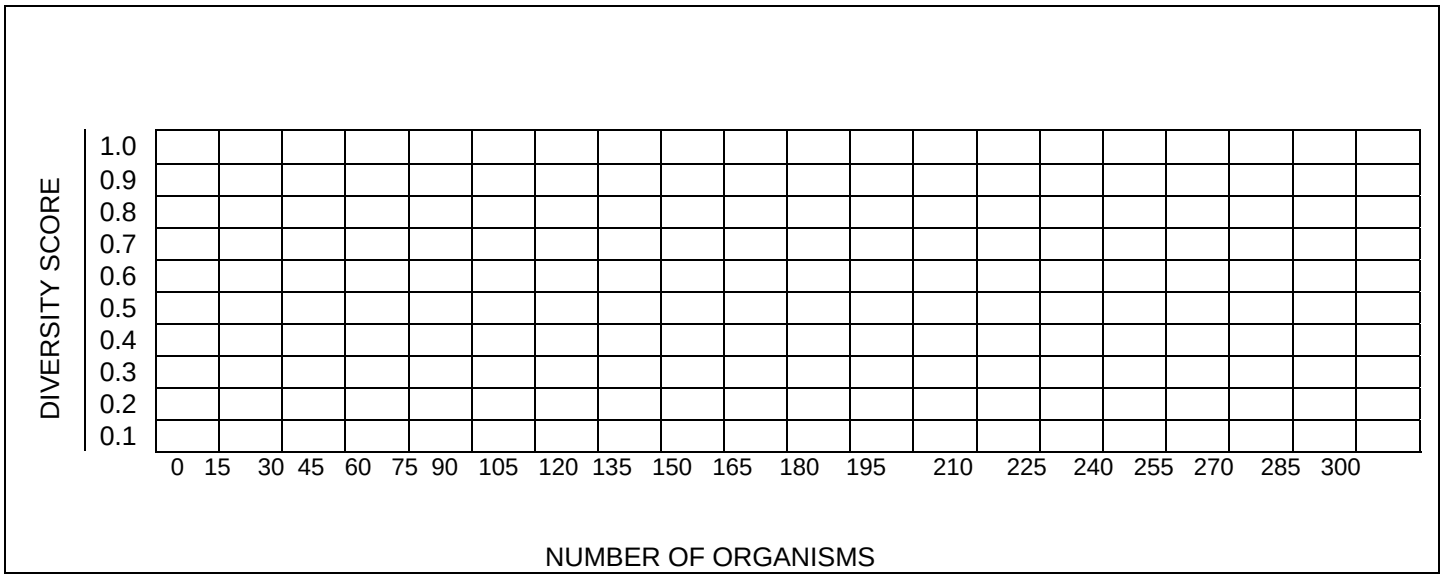
Make a graph of Diversity score Vs Number of Organisms for selection of appropriate Diversity score at a linear range.



Note:If, there is no linearity in the graph, then take the average value of Diversity Score.

BILOGICAL WATER QUALITY CRITERIA (BWQC)

To assess the actual health of water bodies, CPCB has derived a Biological Water Quality Criteria (BWQC) for water quality evaluation. This system is based on the range of saprobic values and diversity of the benthic macro-invertebrate families with respect to water quality. The system has been developed after extensive field trials and calibration on the saprobicand diversity information of different taxonomic groups of benthic animals collected from artificial substratum and natural substratum of various water bodies. To indicate changes in water quality to different grades of pollution .level, the entire taxonomic groups, with their range of saprobic score from 1 to 10, in combination with the range of diversity score from 0 to 1 has been classified into five different classes of water quality (Table 2) The abnormal combination of saprobicscore and diversity score indicates sudden change in environmental conditions.

Table-2

Range of Saprobic Score (0-10)	Range of Diversity Score (0 -1)	Water Quality	Biological Water Quality Class	Indicator colour
7 and more	0.2-1.0	Clean	A	Blue
6-7	0.5-1.0	Slight Pollution	B	Light Blue
3-6	0.3-0.9	Moderate pollution	C	Green
2-5	0.4-less	Heavy pollution	D	Orange
0-2	0-0.2	Severe Pollution	E	Red

CRITERIA FOR BILOGICAL WATER QUALITY EVALUATION

The biological water quality evaluation using benthic fauna can easily be done by combining the observed saprobic score and diversity score and the biological water quality class can be determined through comparing the results with the ranges of Saprobic and diversity score prescribed in Biological Water Quality Criteria (BWQC).

	Range of Saprobic Score (0-10)	Range of Diversity Score (0-1)	Biological Water Quality	Biological Water Quality Class	Indicator Colour
Results:					

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IDENTIFICATION CHARACTERISTICE OF BENTIHC MACRO INVERDTEBRATES TAXA

PLECOPTRA (Stone fly nymph): - Animals of this group are very sensitive to pollution. They are most abundant in cold, flowing waters with plenty of dissolve oxygen. They are normally found in the bottom of boulder, cobble or gravel. The body consists of head, thorax and abdomen.Thorax bears three pairs of jointed legs with two claws or hooks at the end. They have two tails like filaments at the end of abdomen. Their length is up to 2.5 centimetres.

EPHEMERPTERA (May fly nymph):- These animals are also sensitive to pollution, but some of the tolerant species are also available. They are very similar to stonefly nymph. Normally, they have three tails (sometimes two) at the end of abdomen. The legs bear one hook or claw at the end. They can be distinguished from stonefly nymph by the presence of gills on either side of abdominal segments. Their length is up to 2.5 centimetres.

TRICHOPTERA (Caddis fly larvae):- the sensitive forms of caddis fly larvae live in flowing waters and are known for their construction of hollow cases made up of small stones, gravel, twigs, sand grains etc. .Most of them are either attached to the big boulders or rocks or they are without cases. The tolerant species are mostly associated with the animals of highly polluted waters. The caddis fly larvae are characterized by a cylindrical body. The terminal end of abdomen bears two small hooks. They grow up to 4.0 centimetres.

ODONATA (Dragon fly and Damsel fly nymph):-Thepresence of these animals indicates input of little organic pollution in the slow moving or standing clean waters. They are the nymph of adult dragon fly and damsel fly which are powerful fliers and can fly over several hundred kilometres. They can be observed around water bodies, nymph of dragons is robust while damsel is slender with distinct head, thorax and abdomen.

They can be identified by a very distinct spoon – shaped food capturing protrudable device underneath and head. The dragons have pointed extremities at the end of abdomen whereas damsel fly




nymph have three plate like gill filaments.

CRUSTACEA (Prawn and crab, scuds):- The animals of this group are moderately intolerant of pollution. Body is flattened from either side. The animal can be distinguished from the insects by the presence of more than three pairs of legs; the head is fused with few or all the thoracic segments to from cephalo-thorax. The whole body is covered externally by chitinuous cuticle. These animals can be observed in variety of standing and flowing waters, hiding in plants of shallow waters under the debris and organic detritus.

COLEOPTERA (Beetles):- Animals commonly known as water beetles. Beetles belong to the most diverse order of living organisms. Ofthe more than one million species of insects at least one third are beetles. Body typically consists of head, thorax and abdomen. Head bears the chewing mouth parts, one pair of antennae. Thoraxbearing 3 pairs of jointed legs. The wings are modified to form rigid cover or shield on the abdomen.

Different types of beetles can be found in both standing and flowing waters.




Some of them are surface dwellers whereas others are submerged forms. They fulfill their oxygen requirement mostly from atmosphere. They either store air under their wing covers, or capture air bubbles on the fine hairs that cover their legs and stomach area. Adult beetles are tolerant of a wide variety of pollutants.

HEMIPTERA (Bug):-They are commonly known as water bugs. This is a large order. Diversity within the order is high both in terms of structure and habitat. Aquatic bugs live both in running and standing waters. Most of them are surface dwellers. Some of them are sensitive species but most of them prefer





moderately polluted waters. They either carry air bubble while swimming or breathe through abdominal tall like siphon. They can be distinguished from beetles by structure of head which prolonged in to a beak like structure adopted for piercing and sucking fluids from plants or animals.

MOLLUSCA (Snails and Mussels):- The animals of this group of Benthic macro invertebrates are characterized by the presence of a hard calcareous shell and lacking extremities, The snails have a coiled shell and a muscular foot whereas mussels also termed as bivalves are having shells consisting of two halves connected by an elastic hinge.Mollusks are found in almost all kinds of waters and sediments. Some types of mollusks are quite intolerant to pollution, while others are tolerant. Snails which are having gills are






dependent on the level of oxygen in water and thus are sensitive to oxygen depletion. Those snails which are characterized by the presence of lungs can obtain oxygen directly from atmosphere. Thus, they are able to live in water with a little or no oxygen. The snails with gills are identified by right handed opening shells, whereas the snails with lungs can both right and left handed opening shells.

HIRUDINEA (Leeches):- Commonly named as leeches. They look like worms but do not have hairs on their body. The body is segmented which can shrink and extend. They have suckers at both the ends of body. These animals are indicator of highly polluted waters, and can live for several days without oxygen. They are active during night. Usually they can be found under the stones or rocks. Leeches normally prefer shallow waters with lot of vegetation. They are parasite on animals like gastropods, betties, insect larvae, aquatic birds, fish and crabs.

DIPTERA (Chironomous Larvae or Red Worms):- They are very common indicator of highly polluted waters among the diptera group. They represent the largest family of aquatic insects. These species are





tolerant to the organic pollution and found in high abundance in sedimentation areas. These larvae have thorax without segmented lags mostly with proleg or pseudopodia in one or more segment. Two pairs of tubule or branchiae are present at the end of body segment. They live in running waters making tube –like nests).

LEPIDOPTERA:-These are larvae of butterflies and live in running water inside cases/tubes constructed from mud. 

Thorax bears three pairs of legs in addition to five pairs of legs present on entire body. Gill filaments are found on second and third segment of thorax and segments of entire body.

MEGALOPTERA:-Size of mature larvae ranges from 30-65 mm. They have eight pairs of lateral abdominal 

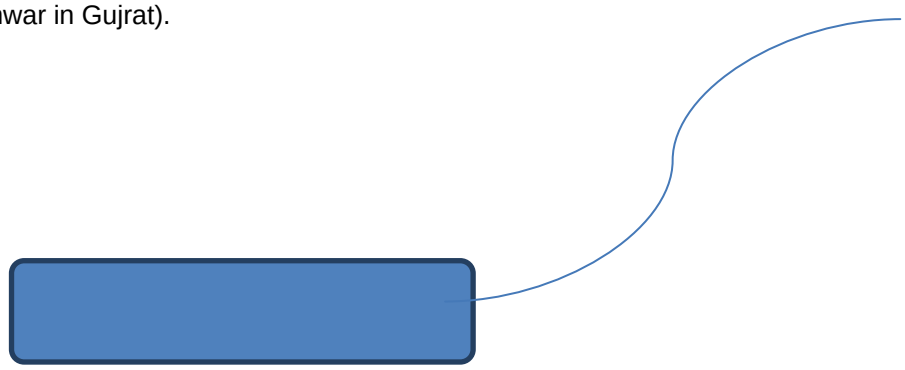
appendages which are unsegmented or imp[erfectly segmented. Anal prolegs are present at the end of abdomen. The can be easily observed due to their large body structure.

NEUROPTERA:-These animals have jointed legs on thorax. Soft prolegs are present on the bach side of the last segment of body. Flashy lobes are present on outer side of each segment and gill filaments are present on the base of each flashy lobe.

OLIGOCHAETA:-They are true worms of which the setae are arranged segmentally. Four groups of setae are often found on each segment of body. Segments are separated at enough distance from

each other and they do not possess legs. Proximal end of body possess a small mouth known as prostomium. Large and fat worms like earthworm, are known as Megadril. Families included in Megadril such as; Moniligasteridae, Microchaetidae,Lumbricidae,Octochaetidae and Megascolicidae. Microdril are always thin and smaller than 10 centimetres. Families of this group of Oligochaeta live in fresh water belong to Tubifiscidae,Naididae and Lumbriculidae.

RAT TALLED MAGGOT: -Rat tailed maggot are typical indicators of severely polluted waters. Rat tailed maggot do not have jointed thoracic legs. Instead of legs, they have many pairs of prolegs, An important feature is the presence of single long or short respiratory tube at the end of abdomen used for breathing at the water's surface. Abdomen is rounded fat body. They are not found in a very high abundance. Their presence indicates very low oxygen content of water. In some waters their presence was observed in abundance (Koteshwar in Gujrat).



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