



AQUASCIENCE: MONITORING FRESHWATER ECOSYSTEMS

Practical activity handbook:

Assessing the health of rivers and lakes



OPhotos by left: C. Medapin and right A. Itareford

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INTRODUCTION

Of the world's water 97.75% is seawater and 2.24% in glaciers; therefore, the freshwater that we rely on for drinking and survival consists of only 0.009% in lakes, and just 0.0001% in rivers. Keeping our freshwaters healthy and clean is therefore vitally important. This activity focuses on river and lakes, the quality of which could be affected by various sources of pollution including effluent from industries (oil and toxic chemicals), non-direct source of pollution such as leakages, sewage, combined sewer overflows, runoff from roads, and agricultural activities (fertilisers and pesticides). Other causes of damage to rivers and lakes can also be attributed to increased urbanization and the construction of dams and canals which increase water flow.

Since water plays a critical role in ecological, domestic and economic activities, it is important that rivers (and lakes) are maintained and regulated. The importance of maintaining clean river systems is now recognized at the national and international level. For example, in the European Union, the Water Framework Directive seeks to ensure that all sources of water are in "good" ecological condition. In England, the Environment Agency monitors the quality of our rivers using the biological indicators that live in them as well as chemical analysis. This activity is designed to give participants a taste of how biological indicators such as invertebrate populations, phytoplankton and zooplankton can be used to monitor freshwater quality alongside chemical parameters such as pH and nutrient levels.

This handbook will introduce methods to sample and evaluate river and lakes using biological and chemical indicators of water quality and ecological status.

1. RIVER ASSESSMENT

Most river pollution is caused by the addition of organic material which is mainly sewage but can be food waste or farm effluent. The organic matter is fed on by bacteria and other microorganisms and these use up the available oxygen. In unpolluted rivers there is a high level of dissolved oxygen, and a wide diversity of aquatic invertebrates. However, as oxygen levels are reduced fewer invertebrate species are able to survive. A **macroinvertebrate** is the term used for invertebrate fauna that can be captured by a 500-µm net or sieve. This includes arthropods (insects, mites, scuds and crayfish), molluscs (snails, limpets, mussels and clams), annelids (segmented worms), nematodes (roundworms), and platyhelminthes (flatworms)

These macroinvertebrates can be listed according to their ability to tolerate these low levels of oxygen, for example, stoneflies and mayflies can only live in high oxygen (unpolluted conditions) while blood 'worms' (actually an insect larva) and tubifex worms can thrive in very low oxygen conditions where other invertebrates are absent.

Benthic macroinvertebrates (i.e. those dwelling on or in the sediments and on macrophytes) form a key component of the freshwater community. Many are herbivorous (plant eating), usually grazing on





unicellular algae or (less commonly) macrophytes attached to the substrate or filtering phytoplankton out of the water. Others feed on dead organic matter (detritus). Some are carnivorous, feeding on other invertebrates and even small fish.

1.1 Assessment River Health using Benthic Macroinvertebrates

We can use the number and type of taxa of macroinvertebrates in a stream as bio-indicators of pollution. Over the past decades, in most of the developed countries, several biotic indices and score systems based on macroinvertebrates have been developed for assessing the ecological status of surface freshwater systems like the BMWP (Biological Monitoring Working Party) score system. This is often used in Europe and adaptations of it have been proposed for other places e.g. Thailand ¹ (see appendix 2 for others). The BMWP score is based on the sensitivity to organic pollution (i.e. nutrient enrichment that can affect the availability of dissolved oxygen). This works by giving each family of aquatic organisms a score from 1 to 10. Those scoring highly (7-10) are sensitive to pollution, and their presence in a river indicates good conditions. Those with low (1-3) scores are tolerant to pollution, and their presence indicate highly polluted conditions.

In this workshop we will use a simplified BMWP score for a river. We take a sample of invertebrates and assign each type using their BMWP scores (see **Recording Sheet**). For each type of organism (e.g., mayfly has a score of 10) we add the scores if we found three **different types** (e.g. 3 types of mayfly with a score each of 10 - they would score 3 x 10 = **30**). We then add up all the scores for each taxon to give a total BMWP score for our sample. However, **we do not take into account the number of individuals** of the same type in the sample – whatever the number, they only score once. We can then use the following **Table 1 of a simplified BMWP for this workshop below** to work out the pollution level in the river or stream.

	ВМШР		ASPT
BMWP Score	Biological Quality	ASPT	Water quality
Over 130	A. Very good biological quality (natural)	Over 7	Very good (natural)
81 – 130	B. Good biological quality	6.0 - 6.9	Good
51 – 80	C. Fair biological quality	5.0 - 5.9	Fair
11 – 50	D. Poor biological quality	4.0 - 4.9	Poor
0 – 10	E. Very poor biological quality	3.9 or less	Very poor

TABLE 1 simplified BMWP score⁺⁺ (from https://oart.org.uk/our-work/projects/water-quality/biological-monitoring)

++Note that here we are using a slightly simplified BMWP score. For a full BMWP score we would have to work out all the different types of invertebrate to a high level of detail (identify to families). This can be very time consuming and needs quite a lot of expertise, so we are going to identify the invertebrates to major groups only.

¹¹ For information on modified BMWP^{THAI} suggested as suitable for Thailand and nearby areas see Mustow, S. Biological monitoring of rivers in Thailand: use and adaptation of the BMWP score. *Hydrobiologia* **479**, 191–229 (2002). https://doi.org/10.1023/A:1021055926316





A problem with the BMWP approach, in common with many other score systems, is the effect of sampling effort, where a prolonged sampling period was required. So overcome this, it has become a common practice to also calculate the **Average Score Per Taxon (ASPT**) value. The ASPT equals the average of the tolerance scores of all macroinvertebrate families found, and ranges from 0 to 10. To calculate the ASPT value you divide the BMWP Score by the number of taxa. The main difference between both indices (i.e. BMWP and ASPT) is that ASPT does not depend on the family richness. ASPT is independent of sample size (being an average) and is less influenced by seasonal variations than the BWMP method and therefore gives an additional, more consistent index.

Health and safety

Collecting invertebrate samples from flowing waters can be dangerous, as there are a number of risks associated with the activity. These include slipping on rocks, being washed away by the currents and drowning. Please wear gloves when handling the water. For general risk assessment see APPENDIX I.

Methods

A. Collecting invertebrates in the field

The most common method of sampling benthic invertebrates in shallow streams is by the 'net kick sampling' technique using a standard pond net. Sampling involves collecting invertebrates on or in the substrate in the net following disturbance with the feet ('kick sample').

- 1. Label the buckets
- 2. If the river/stream, ensure the water level is OK to enter
- 3. Wearing waders, stand in the river facing upstream (i.e. with the river flowing towards you); hold the net in the water with the opening facing upstream
- 4. Now, you are ready to do a 'kick sample'. Set a timer for 3 minutes and vigorously disturb the river substrate with one foot (do not use both feet as there is a risk of falling over). This kicking action will disturb the invertebrates in the river sediments and they will flow into the net. It is recommended you do this in 3x1 minute sessions as is very tiring.
- 5. After kick sampling, empty the net contents into a large tub and add a small quantity of water to cover the contents.
- 6. Rinse the net into the tub to release any attached invertebrates

If necessary, this process can be repeated until enough invertebrates have been collected.

7. In addition, do a 'stone search' by turning over large stones/other substrates and look beneath them. Scrape off any invertebrates into the same tub as the kick sample.





- 8. The kick sample and stone search can be repeated a number of times and at different locations if desired until enough invertebrates have been collected for the purpose of the practical
- 9. Head back to the laboratory.

B. Back at the laboratory

- 1. Sieve and wash away the excess water in the samples. Take a sample (or portion of sample) and place it in one of the large white trays.
- 2. Search through the tray using tweezers and remove all of the invertebrates that are in the sample into petri dishes.
- Sort them into 5 different petri dishes to begin with using the identification keys and pictures. a. Mollusca b. Worms & leeches c. Mayfly, stonefly & Damselfly d. Caddisfly e. mites and others
- 4. For each group count the number of taxa (types), record your data in RECORDING SHEET 1 and then calculate a simplified BMWP and APST score for that group using scores in RECORDING SHEET 1-benthic invertebrates.
- 5. IF you can't ID your samples on the same day you can store your samples in 80% ethanol in vials.
- 6. Note that the *number of individuals* within each group does not matter if you find 1 individual of stonefly type 'A', that scores 10 the same as 8 individuals of type A.

For example, if your sample had

2 types of cased caddisflies =score 7 x 2 = 14

1 type of freshwater shrimp = score 6 x 2 = **12**

3 types of leech = score 3 x3 = 9

A higher score for the Type/Taxa means it is the more sensitive to pollution. So if your final score is high then it indicates that the river is less impacted by pollution.





RIVER RECORDING SHEET 1 – BENTHIC INVERTEBRATES

Drawings from https://www.nrem.iastate.edu/class/assets/aecl518/Data%20and%20Tools/IOWATER advanced benthic_key.pdf

Name	Drawing	Score per type/Taxa (a)	Number of different types (b)	Score (a * b)
Stonefly (Plecoptera)		10		
Mayfly <i>(Ephemeroptera)</i> except Baetidae =4 & Damselflies <i>(Odonata)</i>		10		
Cased Caddisfly (Trichoptera)		7		
Freshwater Shrimp (Amphipoda)	19 Mar	6		
Caseless Caddisfly (Trichoptera)		5		
Flat Worm (Planariidae)	97	5		





Water mite <i>(Acari)</i>	T	5	
Beetle larva (Coleoptera)		5	
Crane Fly <i>(Tipulidae)</i> and Horse Fly Larva <i>(Tabanidae)</i>		5	
Black fly larva (Simuliidae)		3	
Leech (Hirudinae)		3	
Snail (Gastropoda)		3	





TOTAL SIMPLIFIED	BWMP SCORE	
	DWINI JCOIL	

ASPT score (i.e total BMWP score/no of taxa).....

From your score, what is the health of the river: very poor,						
poor, fair, good, or very good?						
(refer to Table 1)						

Suggested link to resources to help ID macroinvertebrates

This website (see link below – accessed April 2021) of The West Virginia Department of Environmental protection has " a wide variety of resources that will help you become more familiar with benthic macroinvertebrates. There are examples of simple field guides, family-level manuals and a wide variety of additional resources."

https://dep.wv.gov/WWE/getinvolved/sos/Pages/Macros.aspx

Also on the next 2 pages is a simplified key showing pollution tolerance for the different groups.

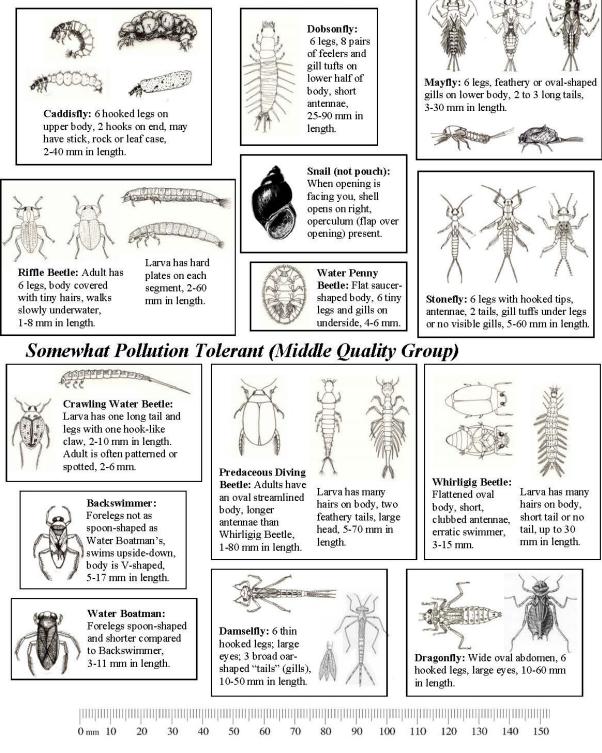




IOWATER

IOWATER BENTHIC MACROINVERTEBRATE KEY

Pollution Intolerant (High Quality Group)

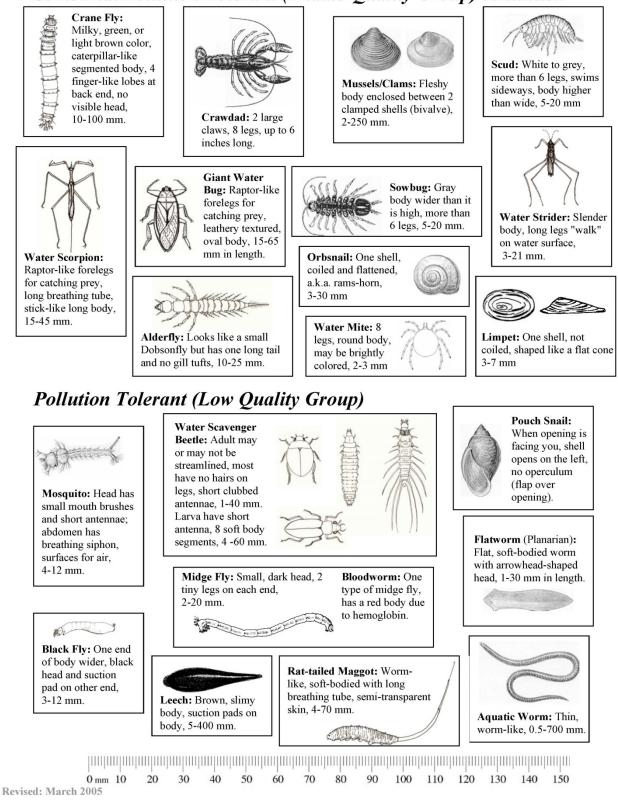






IOWATER

Somewhat Pollution Tolerant (Middle Quality Group) continued







1.2. Assessing River Health Using Chemical Parameters

We will measure a number of parameters of rivers including temperature, pH, electrical conductivity (a measure of the amount of dissolved ions in the water) in the field and also the 3 nutrients phosphate, nitrate and ammonia back in the lab. However, if you have the equipment there are a whole range of parameters that are commonly determined. They are, namely, pH, water temperature (Temp.), conductivity (C), transparency (T), hardness (H), alkalinity (A), chloride (Cl), magnesium (Mg), phosphate (PO), nitrate (NI), iron (Fe), free carbon dioxide (CO₂), dissolved oxygen (DO), biological oxygen demand (BOD) and chemical oxygen demand (COD).

- The direct effects of temperature are due to effects on metabolic activity; many organisms have narrow tolerances to temperature. Indirect effects are due to changes in density and the concentration of dissolved oxygen.
- The pH is important as organisms have defined tolerances to pH. The pH of fresh water varies from around 4.5 in upland peaty catchments to 10.0 in waters with extremely high primary production. However, the most frequently encountered range is 6.5-8.0.
- Nitrogen is an important nutrient and most aquatic plants can only utilise nitrate and ammonia although cyanobacteria are able to use 'fix' dissolved molecular nitrogen. Too much nitrate can induce eutrophication, and result in algal blooms.
- Although ammonia is a plant nutrient it may become toxic to plants and animals at elevated levels, in particular the un-ionised form (NH₃) that predominates at high (alkaline) pH and temperature.
- Phosphate is also a plant nutrient but at high levels can result in eutrophication with excessive plant growth.
- Electrical conductivity is a measure of a solution's ability to conduct an electrical current; the purer the water the greater will be the resistance to electrical current and the lower the conductivity and is proportional to the major cations present (e.g. calcium, magnesium, sodium, and potassium). The conductivity of most freshwater is between 5 to 150 mS/m,

See below for General quality assessments for phosphate and nitrate where 'high' roughly corresponds to EU limits for drinking water and nitrate limits.

If water samples are also collected in the field to take back to the lab for nutrient analysis and then these have to be filtered using 0.45um filters before analysis.

Table 2 General Quality Assessment for Phosphate (& indicates possible eutrophication)

Class	Grade limit (mg P L ⁻¹)	Description
1	<0.02	Very low
2	>0.02-0.06	Low
3	>0.06-0.1	Moderate
4	>0.1-0.2	High⁺
5	>0.2-1.0	Very high ⁺
6	>1.0	Excessively
		$high^+$





Table 3 General Quality Assessment for nitrate

Class	Grade limit (mg NO_3 L ⁻¹)	Description
1	<5	Very low
2	>5-10	Low
3	>10-20	Moderately
		low
4	>20-30	Moderate
5	>30-40	High
6	>40	Very high

The methods for nutrient analysis are varied and we will use an adaption of aquarium/pond water test kits. But there are also other nutrient ion electrodes and lab analysis techniques that could be used instead.

RIVER RECORDING SHEET- CHEMICAL PARAMETERS

River sample	Nitrate absorbance⁺ @546nm	Nitrate (ug/ml)	Phosphate absorbance ⁺ @720nm	Phosphate (ug/ml)	Ammonia absorbance⁺ @685nm	Ammonia (ug/ml)	рН	temp	Electrical conductivity
1									
2									
3									

[* For our technique using water test kits and spectrophotometers – and where detail instruction sheets are provided in the lab - these are the conversion factors:

Nitrate (mg/l) = absorbance/0.026 Phosphate (mg/l) = absorbance/0.81 Ammonia (mg/l) = absorbance/5.01]





2. LAKE ASSESSMENT

We will be also be using the above chemical parameters for our Lake assessments. In addition, we will be also assessing the phytoplankton and zooplankton diversity in the lake instead of macroinvertebrates.

Lakes often contain high pollution levels relative to the surrounding landscapes and environment. Rivers and streams drain pollutants from the landscape where they concentrate in lakes and other water bodies. Pollution affects water quality in lakes and other freshwater resources around the globe. It can take many forms from industrial, agricultural, or municipal sources; a few common examples include pesticides, herbicides, sewage, and litter.

2.1 Assessing Lake Health Using Chemical Parameters

Use same methods as described above as for river water samples.

LAKE RECORDING SHEET – CHEMICAL PARAMETERS

LAKE sample	Nitrate absorbance @546nm	Nitrate (ug/ml)	Phosphate absorbance @720nm	Phosphate (ug/ml)	Ammonia absorbance @685nm	Ammonia (ug/ml)	рН	temp	Electrical conductivity
1									
2									
3									





2.2 Assessing Lake Health Using Phytoplankton

Phytoplankton (i.e., those plants living in the water column) are a key component of standing waters and slow-flowing running waters. They are the major primary producers in standing waters. Phytoplankton are a very diverse group but are characterised by an absence of roots, stems and leaves. Species of phytoplankton are adapted to different physical/chemical conditions, including nutrient status. The trophic status of a lake can therefore be assessed by an examination of the dominant species of phytoplankton (see Table 4). Diatoms can provide a rapid response to environmental changes. Large numbers of cyanobacteria and green algae can indicate high nutrients and eutrophic status.

The presence of **Cyanophyta (blue-green algae)** in a water sample can be a cause for concern, especially when in large quantities as algal blooms. These can form a thick surface layer with major impacts on all other lake inhabitants. Some of these cyanobacterial blooms can be toxic as some Cyanobacteria like *Microcystis sp.* produce cyanotoxins. These frequently lead to the closure of recreational waters and can cause serious illness if consumed. Elevated levels of nutrients from human activities (e.g., nitrogen and phosphorus), warmer temperatures, still water, and plentiful sunlight can promote these harmful algal blooms (HAB). It is important to recognize the presence of an algal bloom in a waterbody and to distinguish a potentially toxic harmful algal bloom from a non-toxic bloom.

Table 4. Example of Dominant phytoplankton in lakes in summer ²

(modified from Bellinger and Sigee, 2010 Freshwater Algae: Identification and Use as Bioindicators. Chichester: Wiley-Blackwell)

Lake type	Dominant phytoplankton
Oligotropic (low nutrients)	Cyclotella
	Rhizoslenia
Mesotrophic	Dinobyron
	Coenococcus
Eutrophic	Anabaena
	Aphenziomenon
	Asterionella
	Closterium
Hypertrophic (extremely high nutrients)	Pediastrum
	Coelastrum
	Oocystis

² Also see......Didem Gökçe (2016) Algae as an Indicator of Water Quality- Open-access peer review chapter. DOI:10.5772/62916 <u>https://www.intechopen.com/books/algae-organisms-for-imminent-biotechnology/algae-as-an-indicator-of-water-quality</u>





Methods

A. In the field

Phytoplankton are collected using a net attached to a rope with **a 50 \mum** mesh size to collect all but the smallest algae that largely form the nanoplankton (5-50 μ m). The net can be pulled horizontally for surface sampling or vertically for sampling a composite of the water column from depth.

- 1. Place the weight should be sitting inside the net, at the end if doing a vertical haul. Unwind a length of rope, about 2-3m.
- 2. Place your foot on far end of the rope you must keep your foot on it to secure it.
- 3. If doing vertical haul slowly lower net in water and pull back up. Do this 3 times.
- 4. If doing a horizontal haul, after a couple of swings over your head, let the rope go, sailing over the water
- As soon as the net hits the water, gently and at a steady rate begin pulling the net into shore. Do not pull too fast or you will not collect any phytoplankton; do not pull too slowly or the net will sink.
- 6. Transfer the contents of the net to a labelled clean plastic container for subsequent examination back at the laboratory.
- 7. If there a lot of Zooplankton, then filter again through a zooplankton net or the zooplankton will eat all of the phytoplankton before you get back to lab!

<u>Health and Safety</u>; when approaching a water bloom, caution and safety procedures should be used to prevent direct contact with the bloom because some cyanotoxins can be absorbed through the skin. However, microscopic amounts shouldn't be a concern.

B. In the lab

If you are not going to look at the samples immediately then either preserve in 80% ethanol or overnight in the cold. To identify the phytoplankton:

- 1. Place a small drop of phytoplankton sample on a microscope slide and cover with a glass cover-slip.
- 2. Examine the sample under a compound microscope.
- 3. Use the simple introductory guide that will be provided during the course. Note that this is an introductory guide only and once you are familiar with its use you should consult the more detailed keys also provided. We will be mainly looking for cyanobacteria in the sample but we have guides in lab to help ID (identify) the diatoms and green algae in our samples if we have time
- 4. Identification sheets will be given out on the day

The features that should be noted are:

- the shape and size of the cells,
- whether they are present as single cells, colonies or filaments,
- colour of pigments (in fresh specimens),
- whether or not chloroplasts are present and if so their number and shape,
- whether the cells are motile or not and finally the nature of the cell wall.





If you wanted to assess the population numbers then for counting the plankton you could transferred into a Sedgwick Rafter counting cell to count the number of individuals. In this workshop we are only looking at the presence and absence.

There are many keys and information on internet to help you ID your phytoplankton to greater detail.

See the next few pages in this handbook for some help with identifying some of the most common phytoplankton you might come across.

Some suggested links to resources for further info and Identification:

- 1. New Zealand's Crown Research Institute <u>https://www.landcareresearch.co.nz/tools-and-resources/identification/freshwater-algae/identifications-guide/</u> (accessed April 2021)
- 2. EPA <u>https://www.epa.gov/cyanohabs/monitoring-and-responding-cyanobacteria-and-cyanotoxins-recreational-waters</u> (accessed April 2021)
- 3. For full guide to identification of Cyanophyta see: US Geology Service https://pubs.usgs.gov/of/2015/1164/ofr20151164.pdf
- Baker, A.L. et al. 2012. Phycokey -- an image based key to Algae (PS Protista), Cyanobacteria, and other aquatic objects. University of New Hampshire Center for Freshwater Biology. <u>http://cfb.unh.edu/phycokey/phycokey.htm</u> 31 Mar 2021.

Green Algae	Diatoms	Cyanophyta (blue-green algae)

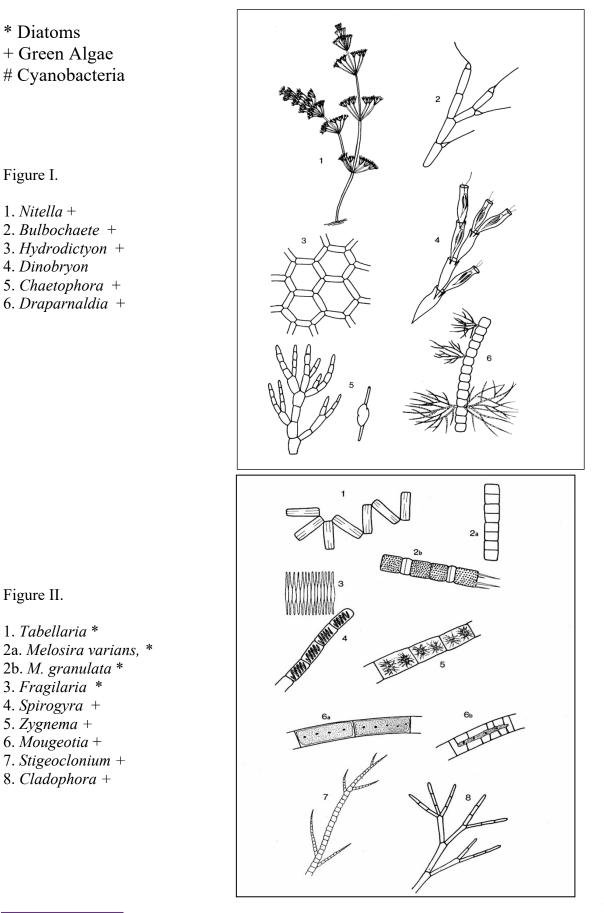
LAKE RECORDING SHEET - PHYTOPLANKTON





The 34 most common Freshwater Planktonic Algae

Thanks to Dr. David Sigee for kindly allowing us to use these illustrations



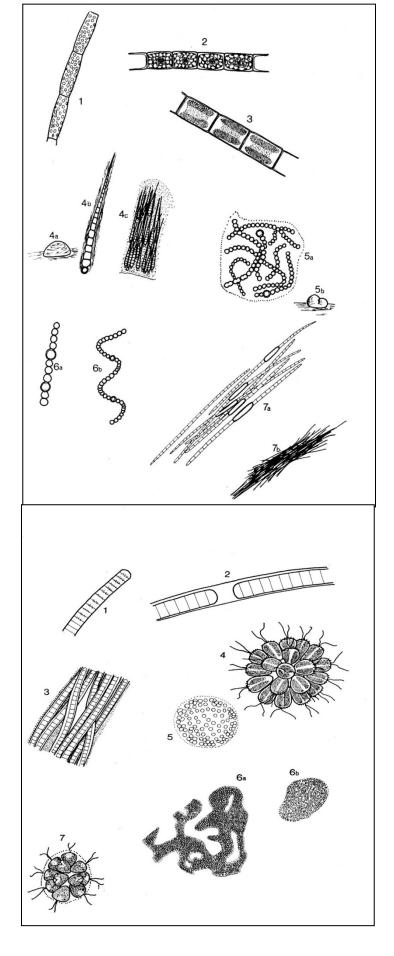




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Figure III.

Tribonema
 Microspora +
 Ulothrix +
 Rivularia # ;
 whole colony;
 trichome;
 section through colony,
 Nostoc #
 Anabaena #
 Aphanizomenon #;
 detail of trichomes;
 raft of filaments.





- 1. Oscillatoria #
- 2. Lyngbya #
- 3. Phormidium #
- 4. Synura
- 5. Coelosphaerium #
- 6. *Microcystis*;#
- a. clathrate colony;
- b. solid colony,
- 7. Pandorina+





MANCHES

Figure V.

Figure VI.

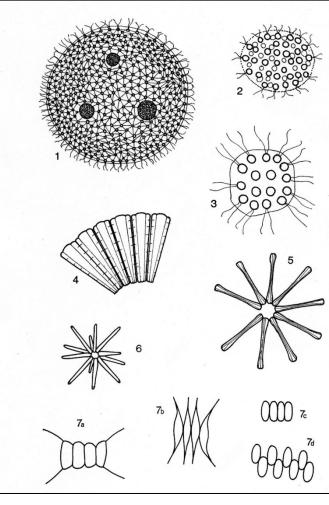
1. Aulacoseira *

2b. P. tetras + 2c. P. biradiatum + 2d. P. obtusum + 3a. Euglena convoluta

3b. E. elastic 4. Ceratium

2a. Pediastrum simplex +

Volvox +
 Eudorina +
 Gonium +
 Meridon *
 Asterionella *
 Actinastrum +
 7a,b,c. species of Scenedesmus +



<image><image><image><image><image><image>



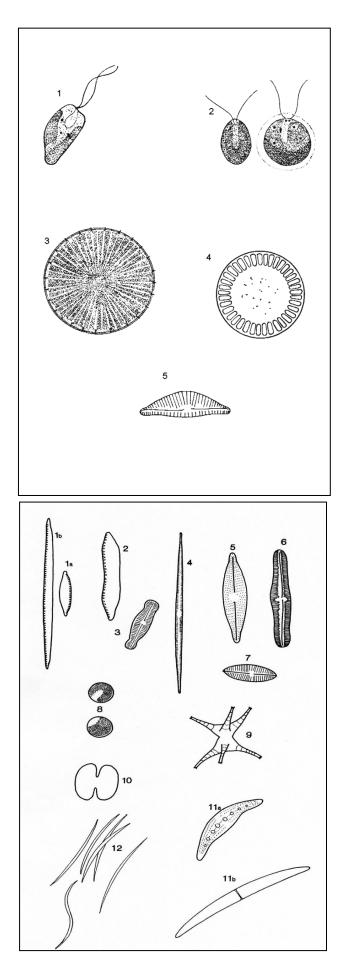


Figure V11

- 1. Cryptomonas
- 2. *Chlamydomonas* +
- 3. Stephanodiscus *
- 4. Cyclotella *
- 5. Cymbella *

Figure VIII.

- 1a,b. species of Nitzschia *
- 2. Hantzschia *
- 3. Gomphonema *
- 4. Synedra *
- 5. Navicula *
- 6. Pinnularia *
- 7. Achnanthes *
- 8. Chlorella +
- 9. Staurastrum +
- 10. Cosmarium +
- 11a,b. species of *Closterium* +
- 12. Ankistrodesmus +







2.3 Assessing Lake Health with Zooplankton

Zooplankton are the uni- or multi-cellular organisms that occupy the water column of lakes and large rivers. Zooplankton provide a biological reflection of the environment and chemistry of the water column. They are consumers of phytoplankton and food for fish. Zooplankton are also important as they excrete nutrients such as nitrogen and phosphorus directly into the water. These nutrients are utilised by phytoplankton and are one of the major controls on phytoplankton growth. Typical zooplankton are Copepods: small, torpedo-shaped crustaceans found in nearly every freshwater habitat, and Cladocerans: small crustaceans commonly called water fleas.

Calanoid copepods are considered oligotrophic, low nutrients indicators. So the ratio number of calanoid copepods to number of cyclopoid copepods frequently declines with increasing eutrophication (Haberman & Haldna, 2014J. Limnol., 2014; 73(2): 263-273).

High abundance of Rotifers indicates that the lakes are eutrophic water bodies.

Methods

In the field:

Zooplankton are collected by in a similar manner to phytoplankton by vertical or horizontal trawls through the entire water. To capture zooplankton you will need to use a $250\mu m$ zooplankton net and preserve 80% alcohol for later identification.

In the Lab:

To identify the zooplankton:

Place a small drop of a well-mixed suspension on a microscope slide using a wide-bore pipette and cover with a glass cover-slip. Or you can put under low power binocular microscopes in petridish.
Identify the main groups - Cladocerans, Calanoid copepod, cyclopoid copepod and Dipteran larva. Note in Recording Sheet how many of each type are present e.g. rare, abundant.

Zooplankton ID guides

UC Davis https://watershed.ucdavis.edu/experiments/zoopsid/ (accessed April 2021)

University of New Hamphire http://cfb.unh.edu/cfbkey/html/choices/rotifera/168/168.htm (accessed April 2021)

Phan Doan Dang, Nguyen Van Khoi, Le Thi Nguyet Nga, Dang Ngoc Thanh and Ho Thanh Hai, 2015. Identification Handbook of Freshwater Zooplankton of the Mekong River and its Tributaries, Mekong River Commission, Vientiane. 207pp. https://www.mrcmekong.org/assets/Publications/tech-No45-handbook-freshwater.pdf





LAKE RECORDING SHEET - ZOOPLANKTON

Туре	example	Absent, rare, common, or dominant
Cladocerans	Brage Førland	
Cyclopoid Copepods		
Calanoid Copepods		
	Per-Otto Johansen	
Copepod nauplius Larva		
Rotifers	Brage Førland	
Typical Dipteran larvae	No. of the second se	





APPENDIX 1: Example of general risk assessment. This should be adapted to suit your sites and risks!

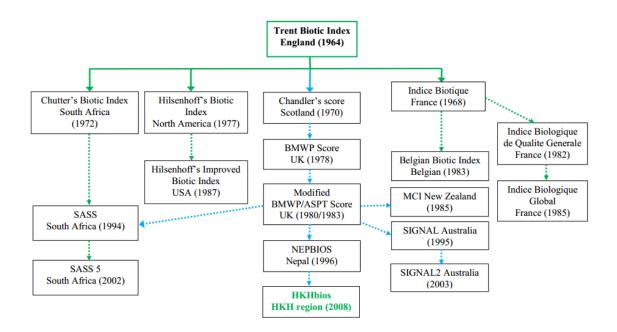
1	Danger from flowing water.	Participants made aware of risk of being near fast flowing water and
		that they should be aware that the rate of water movement can vary.
2	· ·	Participants advised to note depth before entering water, and that risk of sinking into the muddy substrate.
3		Participants advised to avoid ingesting water thrown up by wave action, obstacles or other students. Use gloves.
4	Danger from uneven and slippery terrain and river surroundings	Do not cross or climb on fence including surrounding farms. To be sensible and exercise care. Also, sensible stout footwear is recommended.
5	Danger of thorns, scratches and blisters	Warned of danger. Advised to wear appropriate clothing to protect arms, legs, hands and feet
6	Danger of treading on sharp objects	Warned of danger. Advised to wear appropriate footwear.
7	Risk of drowning	No work to be carried out in the rivers if the water is very deep. The decision of attendant members of staff to be final. No student is to enter water beyond knee-depth. All work to be carried out in company. One member of group to be vigilant for safety of others at all times.
8	Chemicals in the lab	Participants much wear gloves at all times. If spill any then tell staff who will organize clean up. Qualified First aider at hand at all times.
9.	Danger from ingesting cyanotoxins	Participants must not ingest any water samples and wear gloves at all times.





APPENDIX 2: The chronology and relationships of some key biotic indices.

From Dorjii (2016) utility of an existing biotic score method in assessing the stream health in Bhutan. PhD thesis. Queensland university of Techn. <u>https://eprints.qut.edu.au/97993/1/Kinzang_Dorji_Thesis.pdf</u> (accesses April 2021)









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