明愛陳震夏郊野學園
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Field Studies Centre

BIOLOGY

Freshwater Stream

School Name	Student Name	Group
	/	/

Site	Date	Time	Recent Weather Conditions
	/ DD-MM-YYYY /	/	

INTRODUCTION

Background

In Hong Kong, we do not have large freshwater habitats like rivers or lakes, but we do have small streams, ponds, reservoirs, abandoned paddy fields and marshy areas distributed throughout the territory.
Many freshwater systems have been polluted by agricultural, industrial and domestic wastes. It is said that natural water systems free from pollution are getting less and less.

Aims and Objectives

- To appreciate the wonders of the living world.
- To familiarize structure, flora and fauna in a freshwater stream community.
- To observe, compare and contrast the ecology of different freshwater stream habitats.

Equipment

For plant and animal sampling

☐	Aquarium net	×2
☐	Brush	×2
☐	Clip board	×1
☐	Quadrat	×2
☐	Spoon	×2
☐	Trap	×1
☐	Plastic bag	×2
☐	Plastic basket	×2
☐	Plastic box	×1
☐	Plastic tray	×2
☐	Plastic vial	×6
☐	Fresh water streams identification kit	×2
☐	Life buoy with a rope	(Share)

For measurement of physical factors

☐	Compass	×1
☐	Light meter	×1
☐	pH cum thermometer	×1
☐	Dried & weighted filter paper	×2
☐	Filter funnel	×1
☐	Measuring tape	×1
☐	Metre rule	×1
☐	Sampling bottle	×2
☐	Stop watch	×1
☐	Table tennis ball	×1
☐	Towel	×1

Remarks

- Dress in shorts and canvas shoes with adequate tread. Sandals are not recommended. Be careful of broken glass and abandoned construction material etc.
- Walk slowly and try footing for the stability of substratum. Be careful of loose gravel and slippery rock surface.
- Never chase after any fast-moving animals to avoid accidents and habitat destruction.
- Never pollute/damage the environment in all sense. Minimize trampling.
- Minimize disturbance to the local people.
- Team leader should organize members to work in a serious and efficient way. Members should co-operate with the leader.

Since time is limited, you should work efficiently. If you do have extra time, you are highly recommended to carry out your own investigations, provided that it is safe to do so.

1 Site Observation

Choose 10m of the stream and Draw a sketch map top view of the surrounding area on Figure 1, indicating:

- your position in the study site (with a compass) ;
- direction of stream flow ;
- vegetation ;
- breadth, any dam/boulders/trees/fallen log/submerged log etc. ;
- other particulars of interest.

In order to proceed the sampling and measurement works at the same time, divide your group into 2 teams. One is responsible for biotic investigation while the other is to take abiotic measurement. However it is more important to understand the whole picture, so try to get involved in the work of your partners.

Figure 1. Site profile – Top view of the study area

Location of Field Site

Site A

Site B

Past Weather Information

2 Study of Abiotic Factors

Record data on **Table 1&2**

2.1. Water sampling

Carefully collect 2 water samples by sampling bottles (500ml) provided (1 filtered and 1 non-filtered). Mark the sampling site on the sketch map.

2.2. Stream substratum

Use your naked eyes to identify the types of the stream substratum.

2.3. Dimensions of field area

Measure the maximum and minimum width of the area of study by a measuring tape.

2.4 Abiotic factors measurement in quadrants and traps

Use ruler to measure water depth of quadrats and traps.

Use lux meter to measure light intensity at the surface (S) and the bottom (B) of the stream, and calculate the light transmittance.

Use pH meter cum thermometer to measure water temperature of quadrats and traps.

Place ping pong ball at one end of the quadrats or meter ruler, and record the time needs for the ball to flow to another end, and calculate the speed of flow of stream.

★ Do not drop the equipment into the water.

★ Do it prior to other studies!

★ Avoid generating air bubbles. Invert your bottle to check against trapped air and leakage.

Let's think…

Where should the bottle be placed during facing the water flow?

A. In front of your body. OR

B. Behind your body.

Which direction should the bottle mouth face?

A. Against the flow direction. OR

B. As the flow direction.

Table 1.

Texture of substratum	**Gravel / Coarse sand / Fine sand / Mud / Others**
Distribution of substratum	**Homogeneous / Heterogeneous / Patchy / Others**
Water Colour	**Clear / Less Clear / Brown / Black**
Smell	**None / Some / Moderate / Strong**
Floating matters	**None / Some / Plentiful / Abundant**

Table 2.

Sample	Depth (m)	Light Intensity (lux)		Rate of light transmission (%)	Temp (°C)	Drifting time of the ping pong (s)	Current Speed (ms^{-1})
		Above water surface (S)	10cm under surface (B)				
Sampling Area 1							
Sampling Area 2							
Sampling Area 3							
Sampling Area 4							
Trap 1							
Trap 2							

The widest breadth (m)		The narrowest breadth (m)		pH		DO (ppm)	

3 Study of Biotic Factors

3.1. Plant and algae sampling

Search and identify freshwater plants at different microhabitats. Observe any special adaptive features and collect samples with plastic bags. Also collect a full vial of decaying leaves for later investigation.

3.2. Animal sampling (record data on Table 3)

Randomly select 3 sampled area by a 0.5mX0.5m quadrats.

Collect, identify and count the animals at site with the help of nets, plastic tray and brushes. Place the trap in the stream for 30 minutes, and identify and count the animals trapped inside.

★To protect our wildlife and environment, do not collect unnecessary specimen and always put minimal disturbance. Walk slowly in water and always return stones back to original positions.

★Be careful of allergic plant species.

★Perform animal sampling after finishing all other works.

Table 3.

Species Name	Abundance					
	Sampling Area 1	Sampling Area 2	Sampling Area 3	Sampling Area 4	Trap 1	Trap 2
Mayfly Nymph						
Damselfly Nymph						
Dragonfly Nymph						
Stonefly Nymph						
Caddisfly Larva						
Water Penny						
Bloodworm						
Mosquito Larvae						
Pond Skaters						
Long-armed Shrimps						
Predaceous Chub						
White Cheek Goby						
Sharphead Sleeper						
Snail						
OTHERS						
OTHERS						
OTHERS						

Equipment

☐ 250ml beaker	×1	☐ Test tube rack	×1
☐ E. coli detection dish	×1	☐ Wash bottle	×1
☐ Compound microscope	×1	☐ Slides	(Share)
☐ Cuvettes	×2	☐ Cover slips	(Share)
☐ Dropper	×1	☐ Electronic Balance	(Share)
☐ Evaporating dish	×1	☐ Oven	(Share)
☐ Forceps	×2	☐ Heat resistant gloves	(Share)
☐ Glass rod	×1	☐ Dissolved Oxygen meter	(Share)
☐ Petri dishes	×5	☐ 70% Alcohol	(Share)
☐ Stereomicroscope	×1	☐ Spectrophotometer	(Share)
☐ 1 ml syringe	×1	☐ Refractometer	(Share)
☐ Test tube	×2		

Chemicals

☐	Solution A (Ammonium molybdate / H_2SO_4)	(Share)
☐	Solution B (5% Stannous chloride)	(Share)
☐	Solution C (Nessler's reagent)	(Share)
☐	Chemical Oxygen Demand detection kit	×1

4 Biological Investigation

4.1. Plant and animal identification

Use the reference books, photographs and stereomicroscope provided to identify specimens collected from the streams.

★Transfer the animals in the glass chamber specified after identification and clean up the vials.

4.2. Microscopic organisms in algal mass and plant debris

Use forceps to tear a decaying leaf into small pieces. Put the sample onto a slide, observe and record any protozoan and other microscopic organisms present under a compound microscope.

★Put the used slides and cover slips at respective beaker/vial specified.

Remarks

•The equipment/chemicals are rather expensive. Please handle with care and consult technician when necessary.

•Discard all reagents/solutions in the chemical waste bottle.

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5 Water Sample Analysis

Record data on **Table 4**

5.1.Salinity

Read the salinity by placing 2-3 drops of filtered sample onto a refractometer.

★Do not easily discard any solution.

★Remember to rinse the glass chamber before taking readings.

★Fully fill the sample on the glass chamber.

★Prevent generating air bubbles in the chamber.

★Calibration can be made with deionized water.

5.2. Ammonia content

Pour 25ml of filtrate into a 100ml beaker, add 1ml of solution C and swirl the mixture. A yellow colour indicates the presence of ammonia. Fill a cuvette to the white mark and measure the ammonia concentration by spectrophotometer (425nm).

5.3. Phosphate content

Pour 25ml of filtrate into a 100ml beaker, add 1 drop of solution A and solution B respectively and swirl the mixture. A blue colour indicates the presence of phosphate ions. Fill a cuvette to the white mark and measure the phosphate concentration by spectrophotometer (506nm).

5.4. Total suspended solids (TSS)

Weigh a pre-dried filter paper and filter 500ml water sample into another 500ml plastic bottle. Put the filter paper into an evaporating dish, dry in a 105°C oven overnight and reweigh.

TSS (mg/L or ppm) = [increase in mass (mg)] ×2

★Pick up the filter paper with forceps.

★Maximize the surface area of the filter paper during filtration.

★Invert the bottle several times before filtration.

★Rinse the plastic bottle with the filtrate.

5.5. Dissolved oxygen (DO)

Directly measure DO by putting respectively the probes of DO meter directly into the sample.

★Remember to rinse the probe.

★Do not generate air bubbles.

5.6. E. coli content

Use a syringe to spread 1ml water sample from unfiltered water sample onto the E. coli detection dish. Incubate overnight at 37°C.

5.7. Chemical oxygen demand (COD)

Remove the line to clear the aperture from the top of the tube. Press the sides of the tube to expel approximately half of volume. Immerse the tube in the sample. Release the sides to fill the tube up to the half. Shake the tube a few times. After the reaction time (4-6mins) , put the tube on the color chart as shown and compare with the standard colors.

Table 4. Water sample analysis

Salinity (g/100g)	
NH_4^+ (ppm)	
PO_4^{3-} (ppm)	
TSS (mg/L or ppm)	
Dissolved Oxygen (mg/L)	
E. Coli. Testing (cfu/100ml)	
COD (ppm)	

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Discussions and Conclusions

★After pooling all information with other groups, can you draw any conclusions on our study?

★Which factor(s) do you think is/are limiting to the community in the stream? Why?

★Construct a cross sectional profile of your study area after integrating all your abiotic and biotic information.

★Examine the external features of the animals collected, how do they adapt to the environment with respect to their:

- particular microhabitats (beneath stones/free-swimming/on water surface etc.)
- feeding habits (omnivorous/detritivorous/carnivorous/herbivorous etc.)
- feeding relationships (competition / predation / commensalism / mutualism / parasitism etc.)

★Based on the organisms collected or observed, try to construct food chains/web to show the trophic levels of these organisms.

★Draw graphs to show the changes in abiotic factors in different locations along the streams. Do you think the streams have been polluted? Why?

★State the limitations and drawbacks of the investigation. Suggest any improvements for further studies.

References

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Table 1: Physical Factors Analysis (4 point Scale)

Assessment Score / Physical Factors	0	1	2	3
Water Colour	Clear	Less Clear	Brown	Black
Smell	None	Some	Moderate	Strong
Floating matters	None	Some	Plentiful	Abundant
Temp. different from other groups (°C)	< 1	1 - 1.5	1.5 - 2	> 2
Mean rates of light transmission	> 70%	41% - 70%	10% - 40%	< 10%

Mean Assessment Score (A) on Physical Factors Analysis	

Table 2: Chemical Factors Analysis (4 point Scale)

Assessment Score / Chemical Factors	0	1	2	3
Dissolved Oxygen (ppm or mg/l)	> 7.0	5.1 - 7.0	3.0 - 5.0	< 3.0
pH	6.0 - 7.0	5.0 - 5.9 / 7.1 - 8.0	4.0 - 4.9 / 8.1 - 9.0	< 4.0 / > 9.0
Total Suspended Solid (ppm or mg/l)	< 20	20 - 35	35 - 50	> 50
Salinity (%)	< 3	3 - 6	7 - 10	> 10
NH_4^+ (ppm or mg/l)	< 2.1	2.1 - 5.0	5.1 - 7.0	> 7.0
PO_4^{3-} (ppm or mg/l)	< 3.0	3.0 - 6.0	6.1 - 9.0	> 9.0
COD	< 5	5 - 13	14 - 50	> 50

Mean Assessment Score (B) on Chemical Factors Analysis	

Table 3: Micro-organism analysis (4 point Scale)

Assessment Score / Micro-organism	0	1	2	3
Escherichia coli (c.f.u./100ml)	< 180	181 - 400	401 - 610	> 610

Mean Assessment Score (C) on Micro-organism Analysis	

Table 4: Biological Indicators

Organism Group	Indicator Species	
i	Mayfly nymphs Stonefly nymphs Dragonfly nymphs	Water pennies Snails with operculum Microscopic algae (Desmids, Hydrodictyon)
ii	Caddisfly larvae Mosquito larva Snails without operculum	Freshwater shrimps Green algae (Cladophora)
iii	Leeches Blood worm (Chironomus larvae) Protozoa (Paramecium, Vorticella)	Water louse (Asellus spp.) Green algae (Spirogyra, Oscillatoria, Anabaena)
iv	Sludge worm (Tubifex)	Sewage fungi

Table 5: Biological Factors Analysis (4 point Scale)

Biotic Factors / Assessment Score	0	1	2	3
Indicator Organisms	Group i Dominant + ii,iii,iv	Group ii Dominant + iii,iv	Group iii Dominant + iv	Group iv or no organisms found

Mean Assessment Score (D) on Biological Factors Analysis	

Table 6: Degree of Pollution

Average Assessment Score (A), (B), (C), (D)	Pollution Magnitude
0.00 - 0.75	Clean
0.76 - 1.50	Slightly Polluted
1.51 - 2.25	Moderately Polluted
2.26 - 3.00	Severely Polluted

Note: Assumed the Physical, Chemical and Biological factors are equally important.