



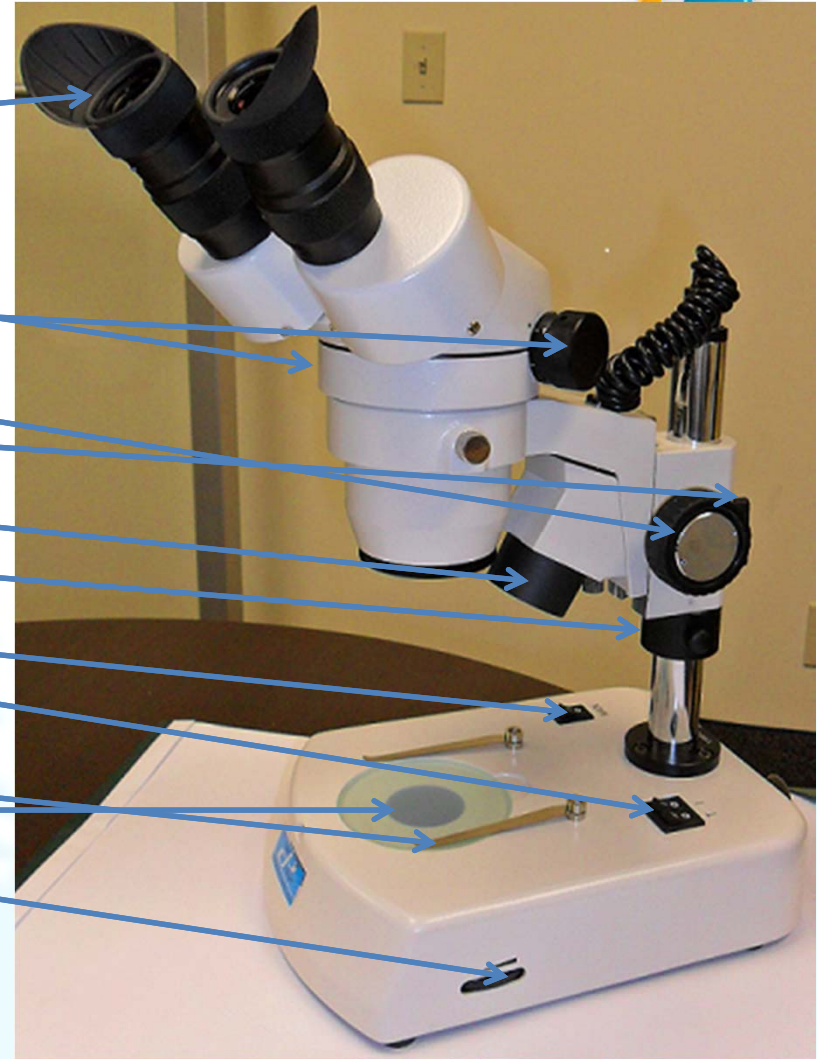
## Microscopes : Parts of them





# Parts of the Microscope

- Eye Piece
- Head Piece
- Magnification Knob
- Course Focusing Knob
- Head Piece Screw
- Upper Light Source
- Locking Collar
- Main Power Switch
- Individual Power Switches
- Stage Clips
- Lower Light Source
- Upper Light Intensity Control





# Introduction to Water Bug Monitoring

Anyone afraid of Bugs?





# Overview

- Introduction
- What are Benthos? and why are they important?
- Aim of the Network
- Fieldwork

## Break

- Invertebrates
- Identification



# Introduction

- Purpose
  - Examining invertebrates for water quality analysis
  - Measuring effectiveness of programs
  - Provide biological complement to Provincial Water Quality Monitoring Program
  - Support the Development of biocriteria for aquatic ecosystem condition
- What it examines
  - Benthic Invertebrates (Bottom feeding Bugs)



# What are Benthos?

- Bottom dwelling aquatic invertebrates
- Include animals like insects, worms, mollusks, crustaceans, and mites



Mayfly of the family Ephemereillidae.



Crayfish or  
Decapoda



# Why are they important

- Abundant and widespread
- Nobody cares
- Easily and inexpensively sampled
- Sedentary (unlike fish)
- Long lived (months to years)
- Many species with different tolerances
- Respond to both water and sediment chemistry
- Readily archived for future reference
- Provide early-warning

Rosenberg & Resh 1993, 1996; Mackie 2001)





# Why is OBBN Important



- Gather provincial data
  - Done by partners who feed their own purposes and participate in collaborative studies
- To share data so that analysis of their own studies can be accomplished because it is a standard that is recognized across the province
- Research



## Aim of the Network

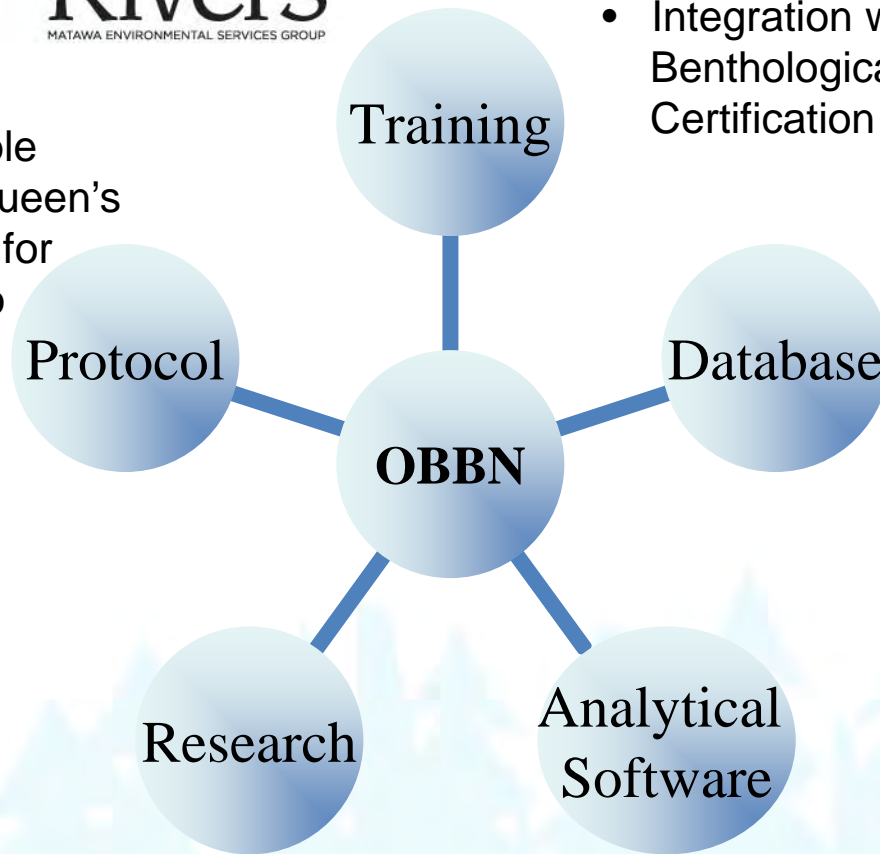
- Gather invertebrates because they are key indicators of ecosystem change
- To gather as much information as possible
- Continue gathering for longer term analysis





# Basis of OBBN

- Available from Queen's Printer for Ontario



- Train-the-trainer
- Integration with North American Benthological Society Taxonomic Certification Program

- New data management system currently under development

- Current focus is on understanding sources of variance and evaluating methods

- Under development
- Will automate control-site selection, summary-index calculation, statistical hypothesis testing, report preparation





# Standardization vs. Flexibility

- The table shows a balance between flexibility and standardization
- Flexibility so that everyone can participate
- Standardization is important to ensure the data collected is comparable between everyone

Biomonitoring Component	Recommendation
Benthos Collection Method	Traveling kick and sweep (where possible); replication in lakes and wetlands, sub-sampling in streams
Mesh Size	500 $\mu\text{m}$
Time of Year	Any season; assessment comparisons use data from the same season
Picking	In lab (preferred) or in field (optional); preserved (preferred) or live (optional), microscope (preferred) or visually unaided (optional); random sub-sampling using Marchant Box (preferred) or "teaspoon" (optional) methods to provide a fixed count per sample
Taxonomic Level	Mix of 27 Phyla, Classes, Orders and Families (minimum); Family (preferred); Genus/Species (optional, recommended for reference sites) <sup>1</sup>
Analysis	Before/after/control/impact (BACI) or Reference condition approach



# Study Design

Design Type	Experimental Control	Experimental Treatment
Before/After/Control/Impact (BACI)	Single reference area, located upstream of the stressor discharge, sampled before and after the discharge begins	Test area downstream of the stressor discharge
Reference Condition Approach (RCA)	Multiple reference sites are grouped by similar biological condition and then matched to the test site of interest	Single test site compared against the most appropriate group of reference sites

The ideal design is the B/A/C/I design. It is meant for pristine locations or minimally impacted areas. If we know these areas are pristine, then we use this design. We achieve the design by doing the following:

- Identify the area that needs to be studied
- Choose sample sites up and downstream from the potential source of contamination
- Continue sampling before and after the development of the source of contamination (develops a larger baseline database sampling before).
- Analyze samples, examine the bugs, sort the species, and count 100 species at random.
- Make comparisons to previous samples.



# Study Design Cont'd

Design Type	Experimental Control	Experimental Treatment
Before/After/Control/Impact (BACI)	Single reference area, located upstream of the stressor discharge, sampled before and after the discharge begins	Test area downstream of the stressor discharge
Reference Condition Approach (RCA)	Multiple reference sites are grouped by similar biological condition and then matched to the test site of interest	Single test site compared against the most appropriate group of reference sites

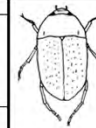
If this site is minimally impacted after the development has been established we would use the RCA. This approach is a little more costly with the amount of minimally impacted sites used to evaluate the test sites. We carry out this design in the following way:

- Find several minimally impacted sites that are similar to the test site.
- Once identified, sample all the minimally impacted sites and test sites.
- Analyze samples, examine the bugs, sort the species, and count 100 species at random.
- Compare all minimally impacted sites to the test site, and note comments.

# Field Sheets

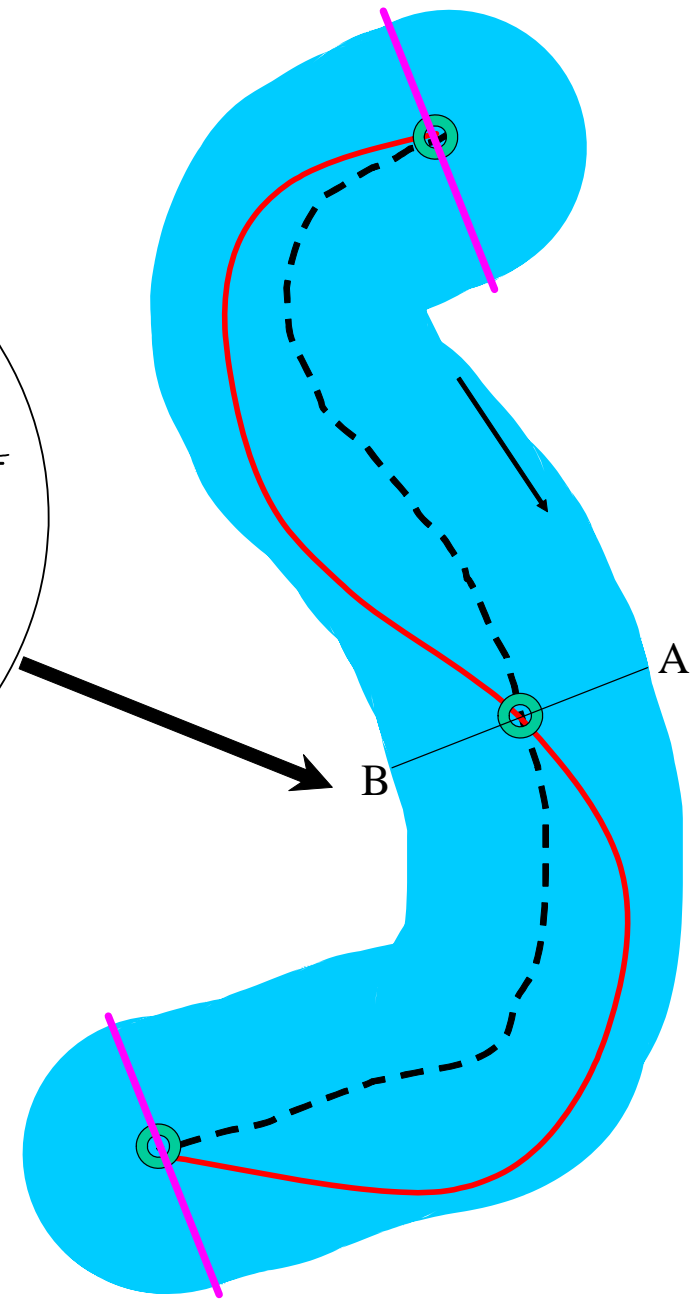
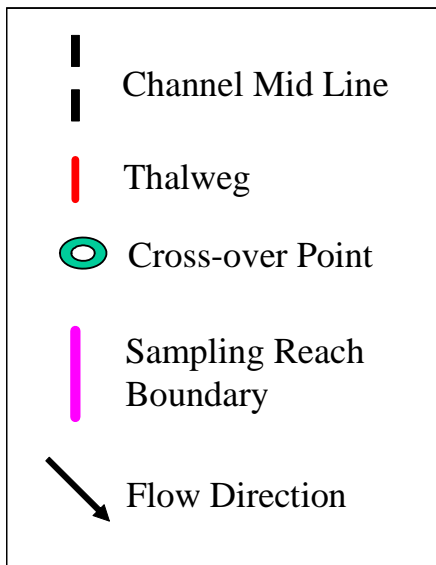
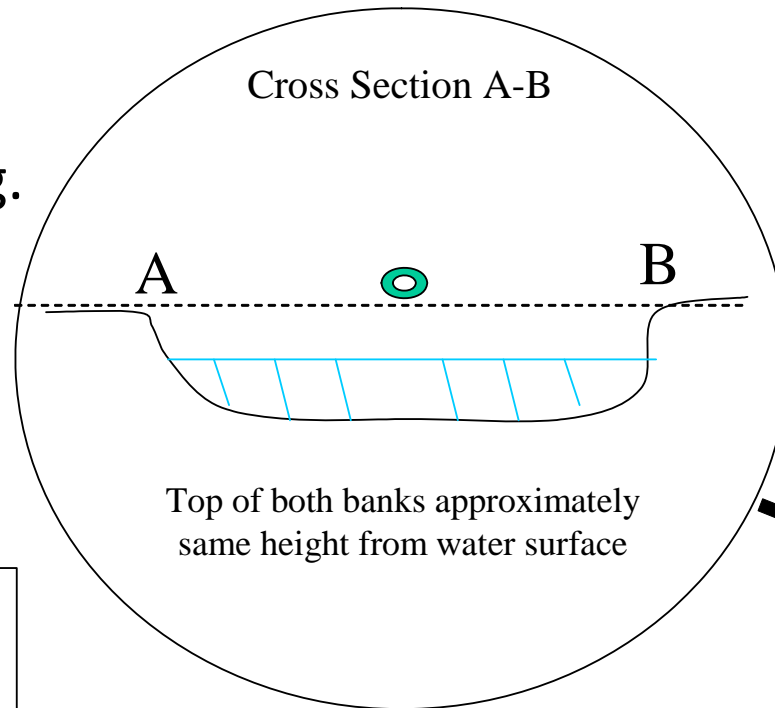
- The sheets are in the handout I have included

Ontario Benthos Biomonitoring Network Field Sheet-LAKES				
Date: _____	Lake Name: _____			
Time: _____	Site #: _____			
Agency: _____	Location: centroid of 3 replicates: Lat/Long or UTM			
Investigators: _____	Elevation (m asl): _____		Datum & Zone: _____	
<b>Water Quality</b> Water Temperature (°C): _____ Conductivity (uS/cm): _____ pH: _____ DO (mg/l): _____ Alkalinity (mg/l as CaCO <sub>3</sub> ): _____				
<b>Site Description and Map</b> <small>Draw a map of the site (with landmarks) and indicate areas sampled. Attach photograph (optional)            Show north arrow.</small>				
<b>Benthos Collection Method (circle one):</b> <input type="checkbox"/> Traveling Kick & Sweep <input type="checkbox"/> Other (specify): _____			<b>Gear Type (circle one):</b> <input type="checkbox"/> D-net <input type="checkbox"/> Other (specify): _____	
Mesh Size: 500 micron (or specify)				
Replicates	Sampling distance covered (m)	Time (min.)	Max. Depth (m)	Location (UTM or Lat./Long; note datum, zone)
Sample 1	_____	_____	_____	_____
Sample 2	_____	_____	_____	_____
Sample 3	_____	_____	_____	_____



# Streams

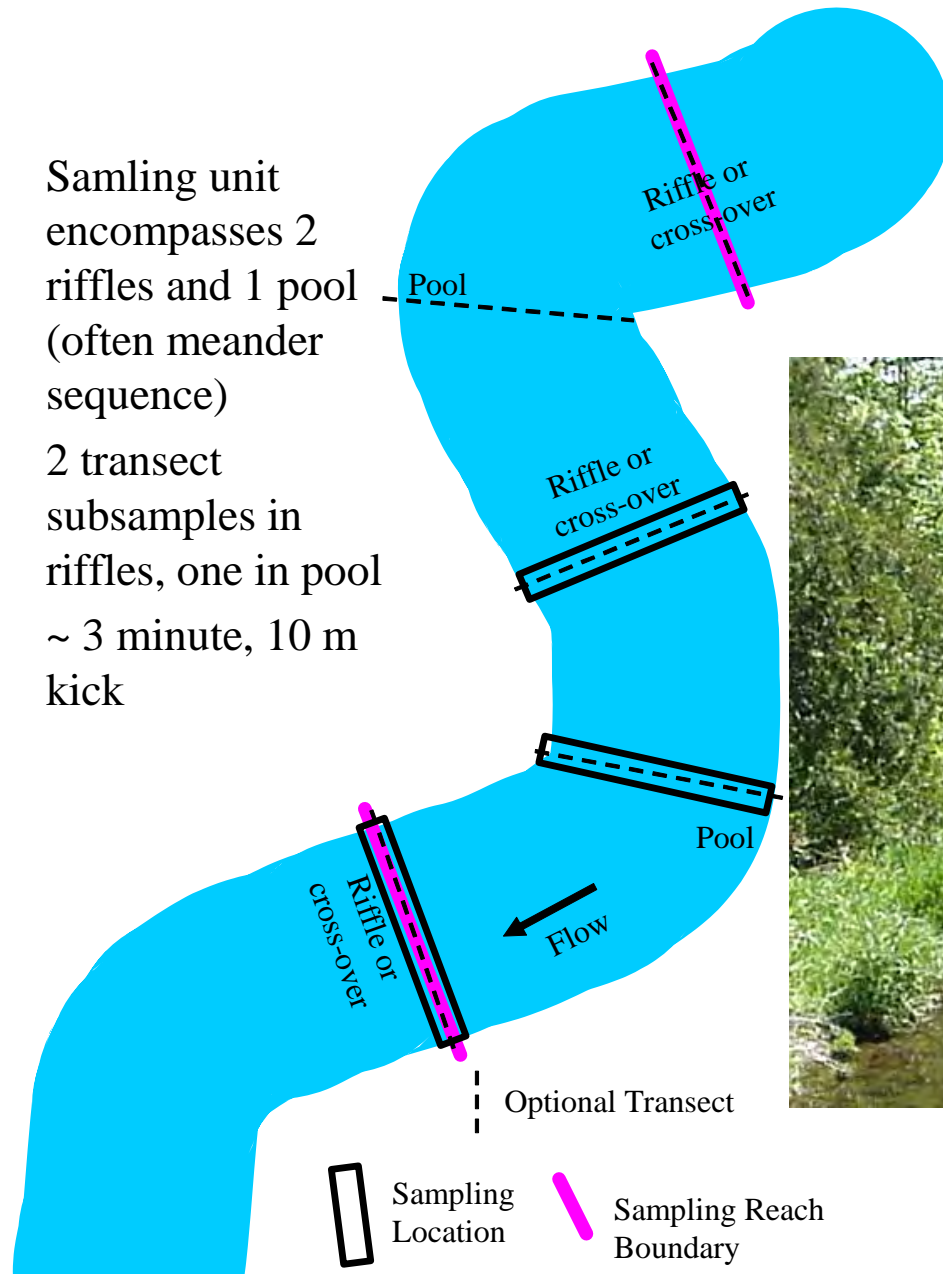
- Sampling unit
- Alternate definitions (pg. 20-21)



# Streams

- Replication & collection methods

- Sampling unit encompasses 2 riffles and 1 pool (often meander sequence)
- 2 transect subsamples in riffles, one in pool
- ~ 3 minute, 10 m kick

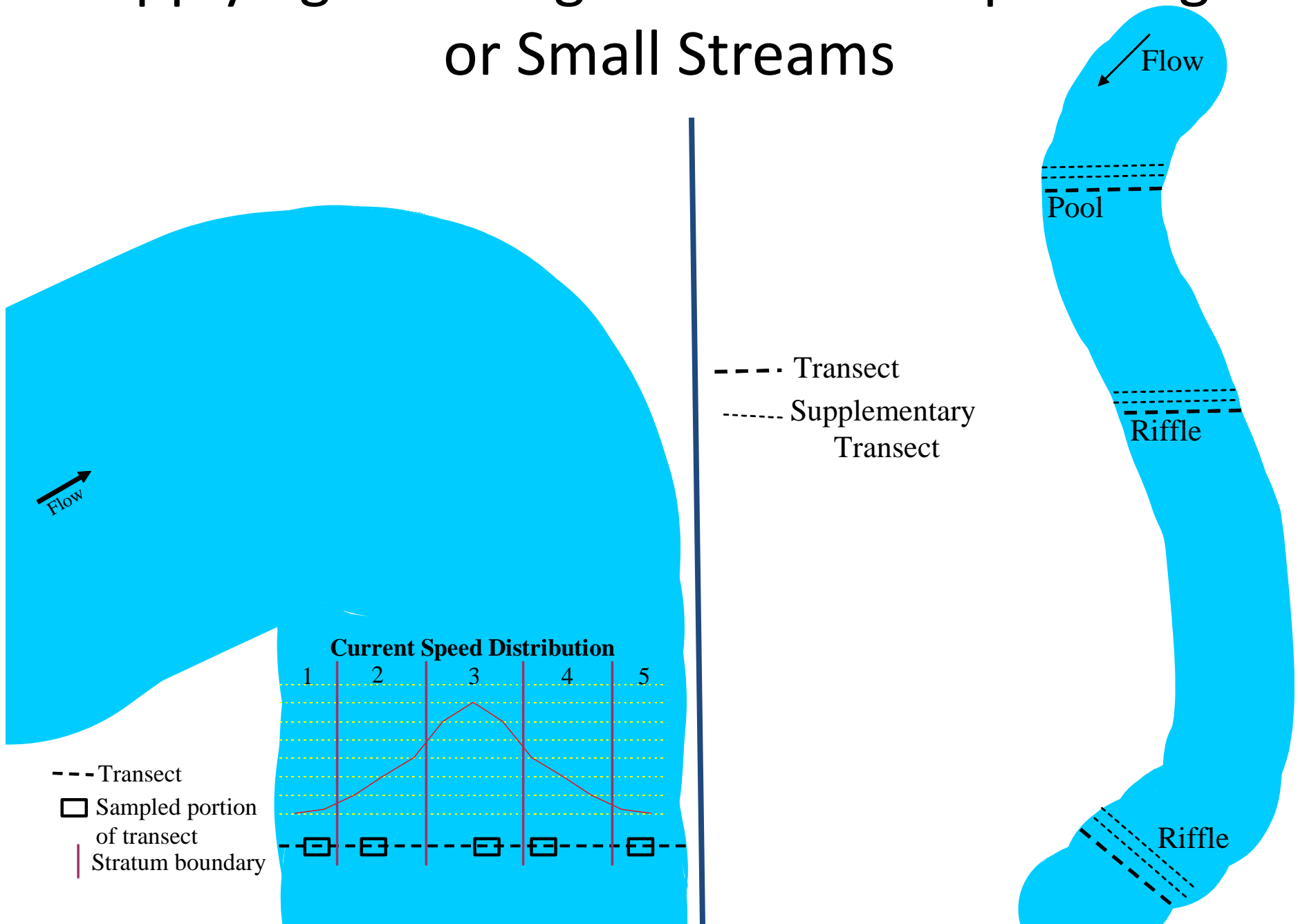


## Transect Traveling Kick and Sweep

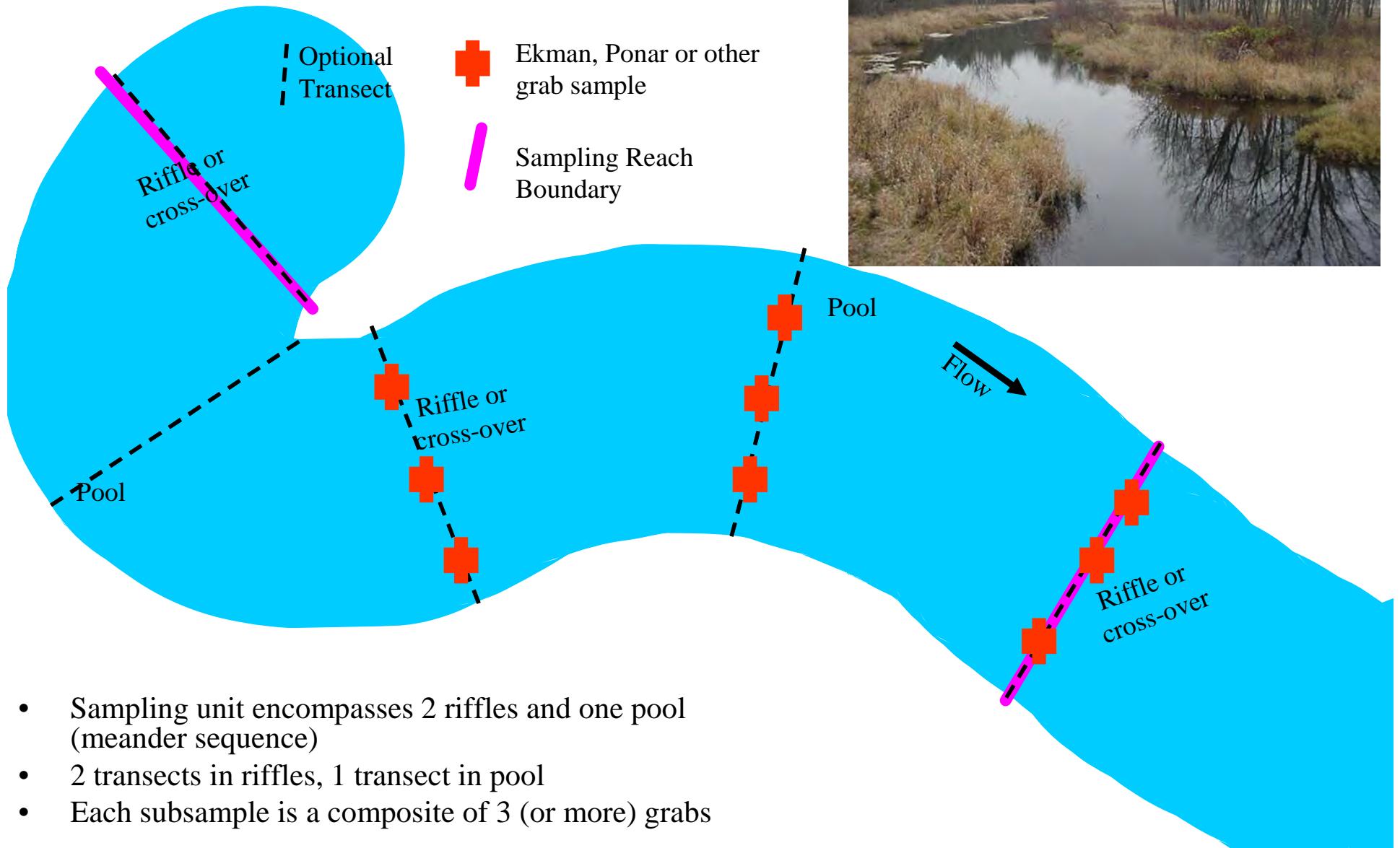




# Applying Traveling Kick and Sweep in Large or Small Streams

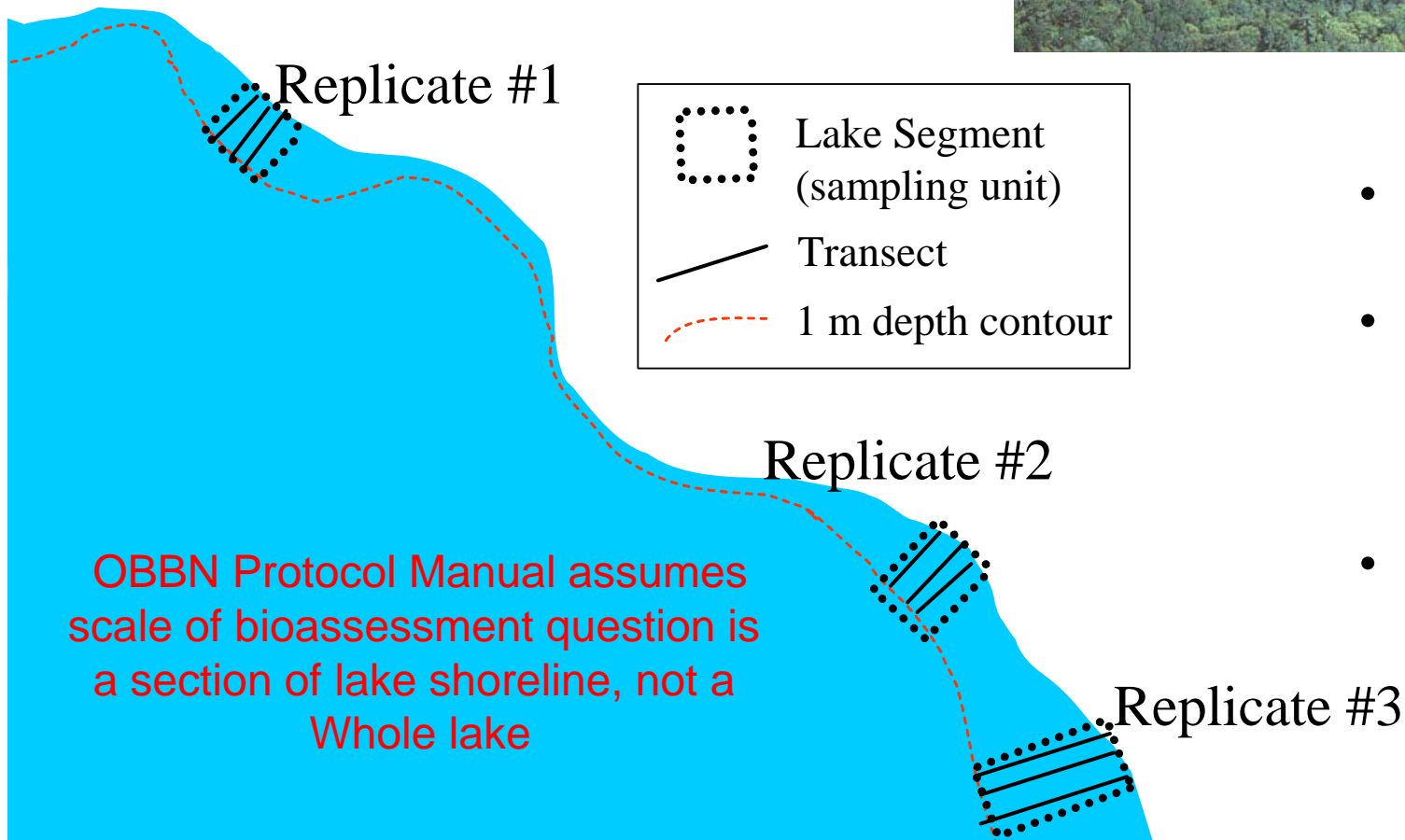


# Streams: Grab Sampling



# Lakes

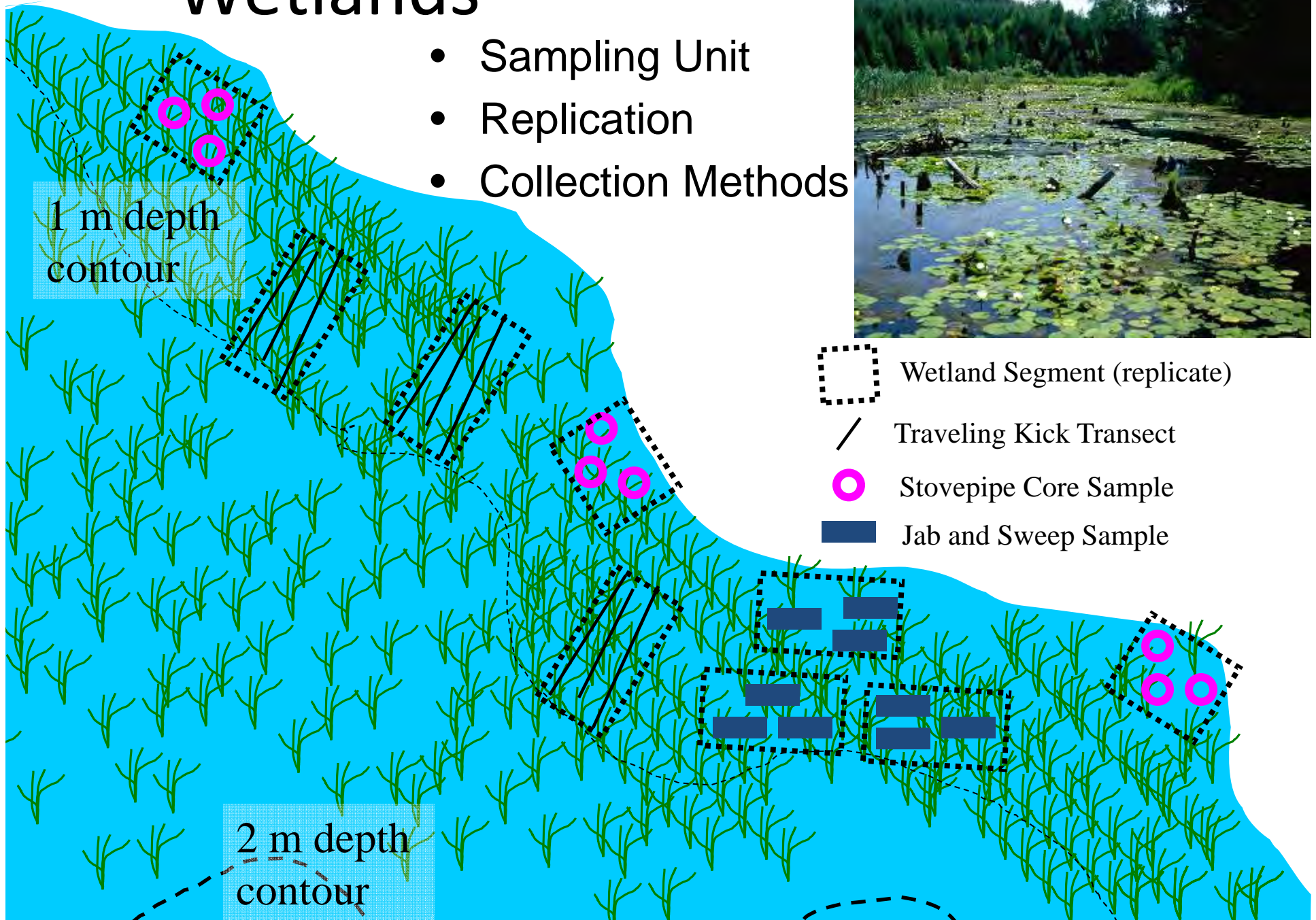
- Sampling Unit
- Replication
- Collection method



- Sampling unit is “lake segment”
- 10 minute traveling kick and sweep along transects
- 3 replicates collected

# Wetlands

- Sampling Unit
- Replication
- Collection Methods



- ⋯ Wetland Segment (replicate)
- / Traveling Kick Transect
- Stovepipe Core Sample
- Jab and Sweep Sample



## Field Work

- We are going to the river to sample for Benthos
- Gear:
  - Chest Waders
  - Clip board
  - 1 bucket
  - Tape Measure
  - PFD's





# Break

Lunch Break at MacGillivray's Landing

Meet back at 1:30 pm





## Questions?

- Anyone have any questions from the first portion of the presentation and outing?
- Has anyone used a Microscope before?



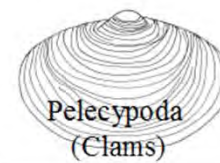
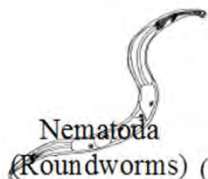
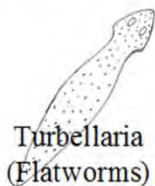
# Benthos ID: 27 Group Level

Water Body Name: \_\_\_\_\_ Site #: \_\_\_\_\_ Replicate #: \_\_\_\_\_ Date (mm/dd/yyyy) and Time: \_\_\_\_\_

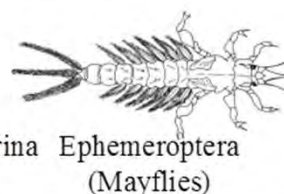
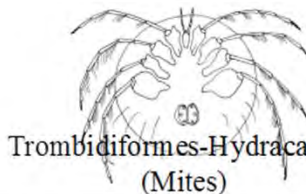
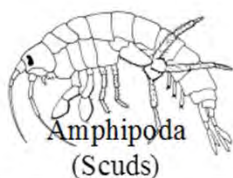
Organization: \_\_\_\_\_ Department \_\_\_\_\_ Address: \_\_\_\_\_

Contact: \_\_\_\_\_ Phone: \_\_\_\_\_ E-mail: \_\_\_\_\_ % picked for 100-count \_\_\_\_\_ # of vials: \_\_\_\_\_

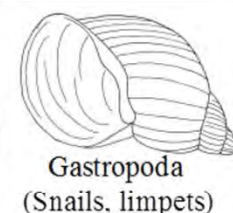
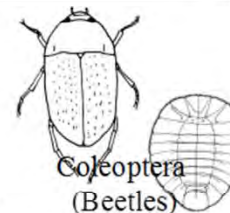
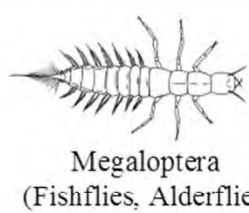
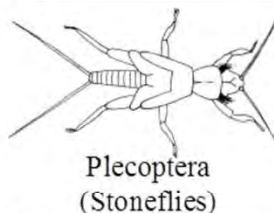
Circle Method: (Sub-sampling) Marchant Box / Teaspoon (Location) Field / Lab (Preservation) Live / Preserved (Magnification) Microscope / Unaided



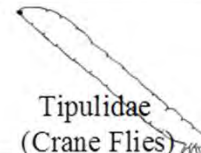
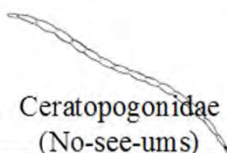
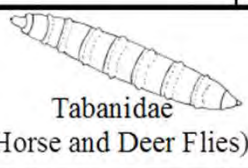
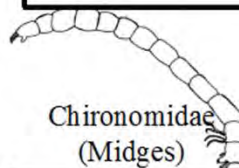
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# Sample Processing

- Sieve
- Sub-sample
  - Marchant Box (preferred)
  - Bucket method
- Sort carefully (microscope preferred)
- Identify and tally (taxonomic level matches training; Family or more detailed preferred)
- 100-count (minimum)
- Preserve and archive sample



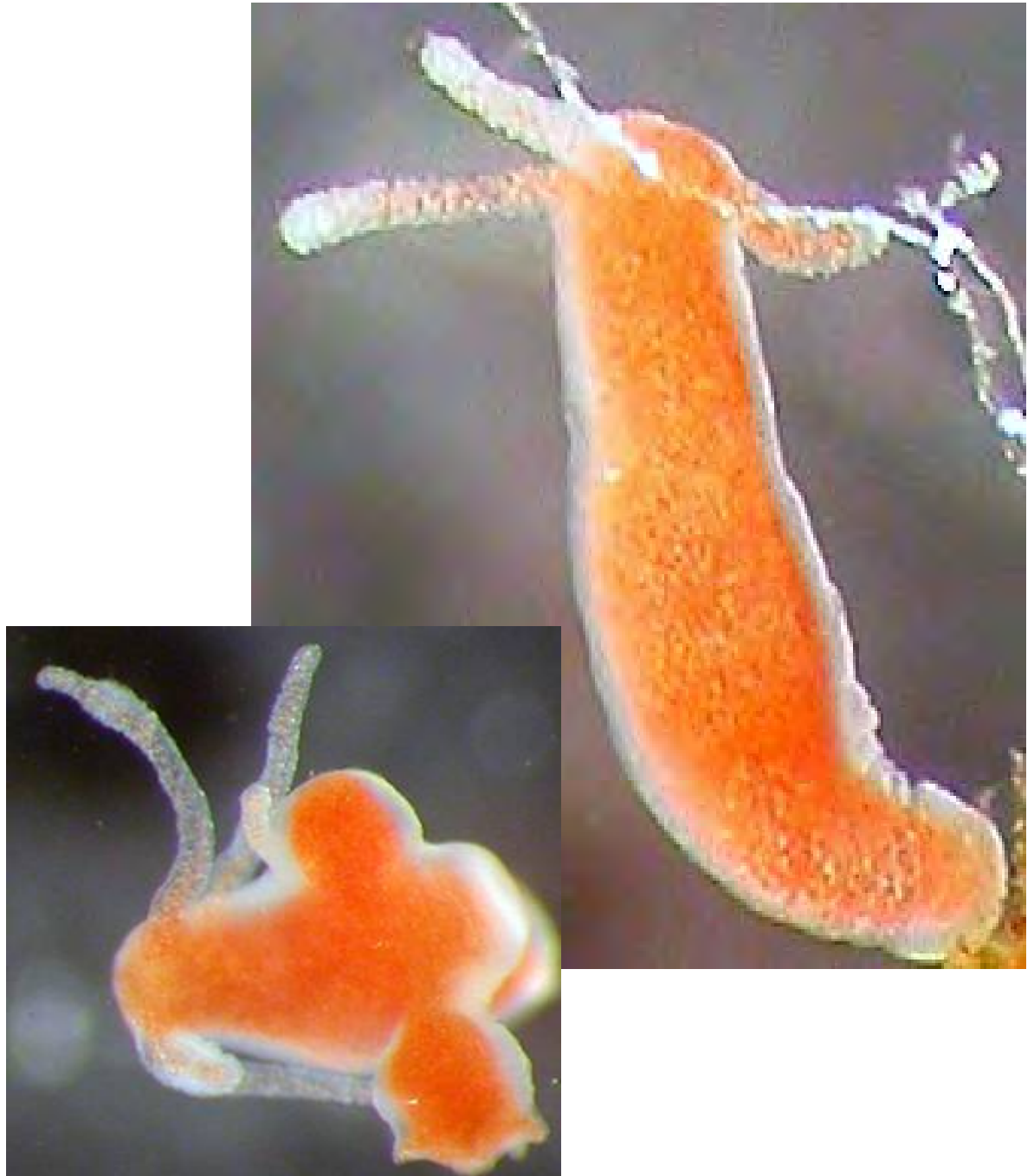


# Sample Processing Tips: Sub-sampling & Picking

- Need random sub-samples
- 100-count but sort entire last sub-sample
- Consider suction device if using Marchant Box
- If using Bucket Method, estimate portion picked by weight or volume
- A bit of soap will sink floaters
- Screen for fast moving
- Sort thoroughly

# Coelenterata (Hydras)

- Inconspicuous, 2-25 mm long polyps
- Hollow cylindrical body, closed and attached at one end, and opening at the other by a central mouth surrounded by tentacles armed with nematocysts
- Asexual reproduction by budding
- Colour variable



# Platyhelminthes (Flatworms)

- 5-20 mm long, Ventral mouth; may have pharynx
- Ciliated epidermis
- Unsegmented
- Triclads:
  - Variable colour
  - Flat (other groups aren't)
  - Heads with eyespots
  - Non-swimmers
  - Creep slowly on bottom of sorting tray



<http://www.microscopyu.com/moviegallery/pondscum/platyhelminthes/dalyellia/>

# Nematoda (Roundworms)

- Unsegmented worms with body cavity and complete digestive tract
- Usually tapered at both ends
- Most <1 cm long
- Longitudinal muscles permit side-to-side bending movements
- Covered by cuticle
- May swim with rapid, whip-like movements
- Pale, usually translucent



<http://www.msmedia.com.au/Medical/NematodL.htm>

# Oligochaeta (Aquatic Earthworms)

- Bilaterally symmetrical, segmented coelomates; round, soft, muscular and elongate
- Like earthworms, but smaller, and have simple body wall and unspecialized digestive tract
- Four bundles of hairs (chaetae) on each segment behind the first
- 1 to 30 mm long
- All aquatic oligochaetes have red blood pigments (aid  $O_2$  uptake and transport)
- May crawl along bottom of tray but often coiled up



# Hirudinea (Leeches)

- 34 annulated segments
- No chaetae
- suckers at both ends
- Ventral genital pores
- Head often with several pairs of eyes
- ~5mm-30 cm long
- Move by inching along or swimming
- Colour varies, brown, olive and black common; typically patterned dorsally



# Isopoda (Sow Bugs)

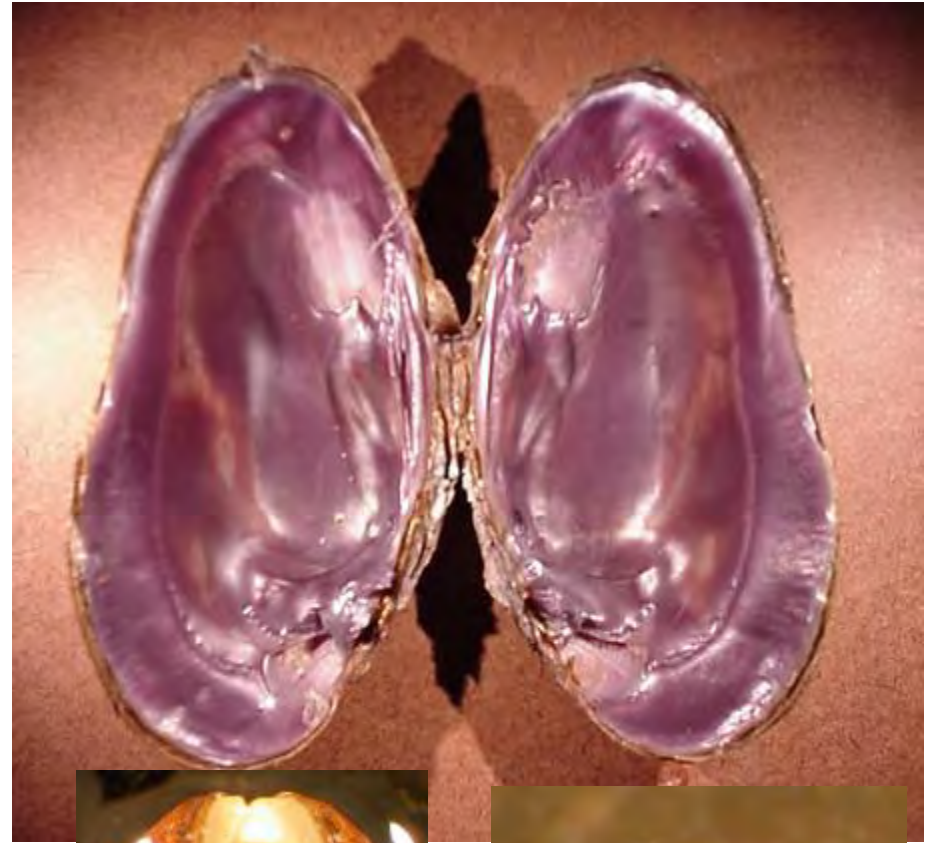
- Dorso-ventrally compressed; 7 pairs of legs, adapted for crawling (first pair sub-chelate, others with simple claws)
- 1<sup>st</sup> antennae longer than 2<sup>nd</sup>
- Usually gray in colour
- Often associated with organic matter
- Uropods bifid
- 5-20 mm long; mini armadillos





# Bivalvia (Clams and Mussels)

- 2 - 250 mm; colour variable
- Hard oval bivalved shell
- Locomotion with muscular protrusible foot
- Found in bottom of tray in sand or gravel
- Watch for empty shells



# Amphipoda (Scuds)

- Laterally compressed
- 2 Long antennae of approx. equal length
- Thorax with 7 pairs of walking legs
- 6-segmented abdomen
- Usually a translucent grey or light brown
- 5-20 mm long, colour variable
- Catch with small piece of screen



<http://www.usask.ca/biology/skabugs/Candlelakebugs/CLcrustacea/gammarus.JPG>

# Decapoda (Crayfish)

- 1 to 15 cm long, often green, brown, blue
- Look like small lobsters; front half of body cylindrical with a hardened carapace; 6-segmented abdomen is dorso-ventrally flattened
- 5 pairs of walking legs: first 3 pairs chelate (claws of forelegs enlarged)
- Hard-shelled, eyes on stalks
- Broad telson used in backward escape
- 1 pair of antennae longer than other pairs



# Hydrachnidia (Mites)

- Body without visible segments (fused cephalothorax and abdomen); 1-7 mm
- Anterior finger-like, 5-segmented pedipalps; simple eyespots; no antennae
- Adults with 4 pairs of segmented legs (larvae with 3)
- Often brightly coloured (red, green, blue, brown)
- Uncoordinated, scrambling swimming motion
- Size, shape, position, and degree of fusion of sclerites are key taxonomic characters



# Anisoptera (Dragonflies)

- Larger and heavier bodies than mayflies; No visible external gills; Modified labium for catching prey
- Big head and eyes
- Often flattened; Jet propulsion
- 15-45 mm; drab colours, often green to greenish brown



# Zygoptera (Damselflies)

- 10 to 22 mm long, drab colouration
- Bodies more tubular, thinner than dragonflies
- 3 gills at terminus of abdomen
- Same modified labium as dragonflies





## Plecoptera (Stoneflies)

- 6-50mm, yellowish, brown or blackish
- Resemble mayflies but with 2 tails and gills never dorso-lateral on abdomen
- Tarsi with 2 claws

# Hemiptera (True Bugs)



- 15-40 mm
- Sucking mouth parts (rostrum)
- Often two pair of membranous wings
- Often with well developed breathing appendages





# Megaloptera (Fishflies, Alderflies)

- Large: 25-90 mm long
- 7 or 8 pairs of lateral abdominal filaments
- Well developed mandibles
- Often with anal prolegs
- Some Corydalids have posterior spiracles with respiratory tubes



# Trichoptera (Caddisflies)

- 2-50 mm long, head and thorax compressed into anterior portion of body
- Dorsal thoracic plates variously sclerotized
- Anal prolegs with hooks
- Often build portable case or fixed retreat



# Lepidoptera (Aquatic Moths)

- Head with ring of ocelli
- 3 pairs of short, segmented, thoracic legs
- Ventral, abdominal prolegs
- 10 - 25 mm, crawl like a caterpillar



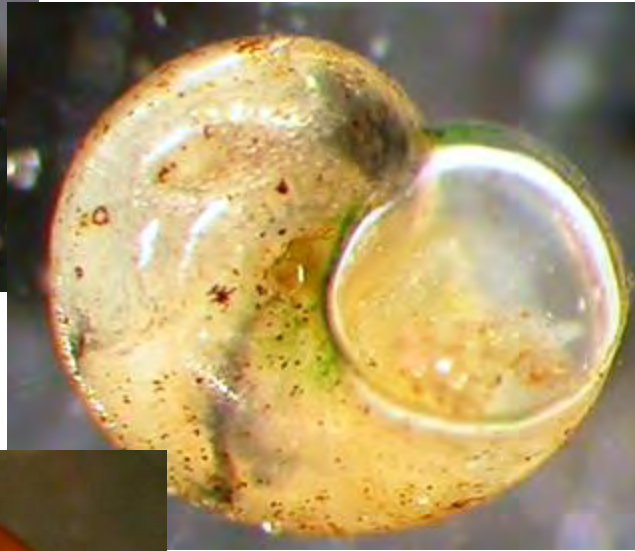
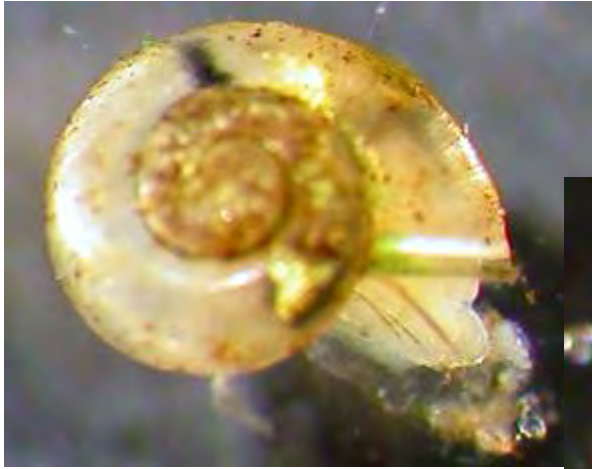
<http://www.nature.ca/rideau/b/b5b-e.html#synclita>



# Coleoptera (Beetles)

- 2 - 20 mm
- 3 pairs of thoracic legs
- Adults: Fore-wing modified as elytra, Antennae with 11 or fewer segments
- Larvae: Sclerotized head with mandibles, maxillae, labium and 2- or 3-segmented antennae; May have unsegmented terminal abdominal appendages





# Gastropoda (Snails, Limpets)

- Soft-bodied, unsegmented animals with a muscular foot, head, visceral mass and shell-secreting mantle
- Radula
- hard spiral or cap-shaped shell; May have operculum
- 2 - 70 mm

# Chironomidae (Midges)

- 2 - 30 mm long, red, white, olive or yellowish
- Well developed, sclerotized head with eyes; anterior and posterior parapods with hooks
- Characteristic shape like letter “J”
- May be in a tube made of fine dirt particles
- Often caught in surface film



# Tabanidae (Horse Flies, Deer Flies)

- 15 - 40 mm
- Pointed at both ends, leathery texture
- Segmented, straight or slightly curved
- Head retracted into thorax
- Several pairs of creeping welts (with tiny hooks) on each abdominal segment



# Culicidae (Mosquitos)

- 3-15 mm
- Fused thoracic segments
- Posterior-dorsal respiratory siphon





# Ceratopogonidae (no-see-ums)

- 3-13mm
- Body may have dorsal tubercles and/or setae
- Commonly:
  - Very slender, pointed at both ends; white, shiny, naked, segmented integument; small pointed sclerotized head
  - No abdominal appendages but may be a tuft of terminal abdominal hairs
  - Remain stiff when picked up with forceps
  - Move by “whipping”



<http://www.junglewalk.com/popup.asp?type=v&AnimalvideoID=3160>

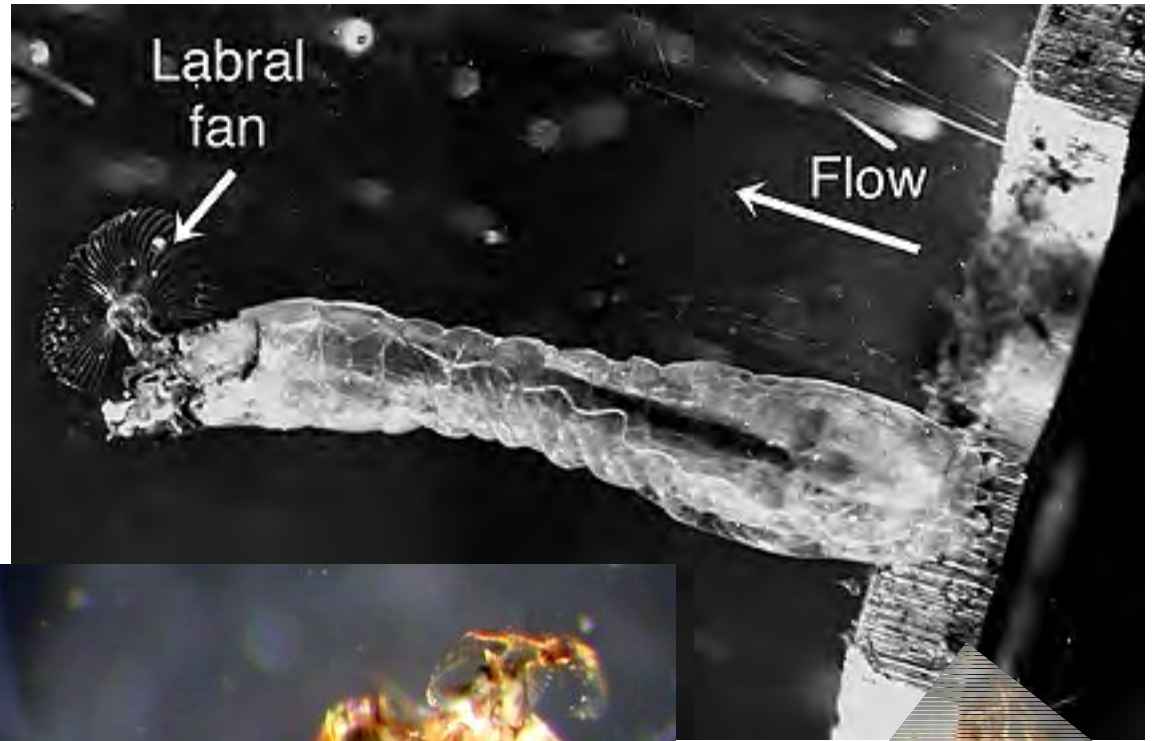
# Tipulidae (Crane Flies)

- 10-50 mm, white, yellowish or brown
- Posterior portion of head capsule incompletely sclerotized and withdrawn into thorax
- Membranous body; may have creeping welts
- Posterior respiratory disc with spiracles and various lobes



# Simuliidae (Black Flies)

- 3 - 15 mm, brown or greyish colour
- Big head, often with labral fans
- Single anterior ventral proleg
- Like flattened maggot with one end 1/3 fatter
- Sessile with posterior attachment organ
- Move with looping (inch-worm) movements



# Misc. Diptera (Misc. True Flies)

Adults with single pair of wings

May have parapods, pseudopodia, creeping welts or other appendages, but **no jointed thoracic legs**

- Often maggot-like; head may be retracted into thorax





Thank you very much!!!

If you have any questions contact me at:

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or

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