REFERENCE MANUAL FOR UNDERGRADUATE INTERNS

2011-2012

Streams Project Internship 2011-2012

Manual Contents

Section 1: Site & Habitat
Section 2: Stream Site Information
Section 3: Water Quality
Section 4: Macroinvertebrates
Section 5: Data and Analysis
Section 6: Field Safety
Section 7: Payroll Instructions
Section 8: Due Dates and Deadlines
Section 9: Independent Project

About this manual:

- Become familiar with it at the outset of your internship.

- Use the two sections in the back of the manual to keep track of your research:
  - Due Dates and Deadlines
  - Independent Project

- Take notes on the data sheets we give you during training week and place them in the back of this manual so you always have extra data sheets handy.

- Use this in conjunction with the Streams Project website (uvm.edu/~streams) which hosts a wealth of additional resources:
  - a searchable PDF of this manual
  - data analysis tutorials
  - mapping and site information
  - searchable database
  - links to useful websites
  - presentation and symposium information

Email streams@uvm.edu if you need assistance. Your message will be directed to the appropriate staff member.

Section1: Site and Habitat

Contents

Site and Habitat Assessment General Info..2

Site and Habitat Assessment Field Checklist..3

Stream Site General Assessment Data Sheet 4

Habitat Assessment Data Sheet (2011-2012)...7

Habitat Assesment & Physicochemical Parameters……………………………………………………......10

Site and Habitat Assessment General Info

There are several components to site and habitat assessment:

- Determining your site's way points
- Naming your site
- Stream Site General Assessment
- Habitat Assessment

Undergraduates: Complete a "Stream Site General Assessment Data Sheet" and a "Habitat Assessment Data Sheet" for each site that any Streams Project researcher investigates, regardless of how many or how few times you sample that site.

High School Teams: Fill out the "Stream Site General Assessment Data Sheet" and a "Habitat Assessment Data Sheet" once for each of your two stream sites.

All Participants: Consider revisiting the Site and Habitat Assessments if you notice major changes in your stream site.

Site and Habitat Assessment Field Checklist

- Data sheets
- Pencils and permanent marker
- Camera
- GPS
- Topographic maps/aerial photos
- Meter tape
- Meter stick
- Plant identification keys

Stream Site General Assessment Data Sheet

2011-2012

STREAM NAME:	TOWN:
DATE:	LATITUDE:
TIME:	LONGITUDE:
STREAM GRADIENT (HIGH OR LOW):	RIVER BASIN:
SITE DESCRIPTION:	INVESTIGATORS:

Stream sketch: On your sketch, note features that affect stream habitat, such as: riffles, runs, pools, ditches, wetlands, dams, riprap, outfalls, tributaries, landscape features, logging paths, vegetation and roads.

Watershed features	Location of stream headwaters (Town name): Predominant Surrounding Landscape: (circle one) Forest Field/Pasture Agricultural Commercial Residential Industrial Other (If other, please specify) Local Watershed non-point pollution (circle one): No evidence Some potential sources Obvious sources Please explain:
Stream Reach Characteristics *Enter from GIS Assesment Report if not measured in the field	Bank full width (meters): Reach length (meters): Channelized? ___ *Upstream Dam: if Yes, ___ km upstream from site Other modifications: Bridge: Within Reach: Yes or No *Upstream: Yes or No if yes, how far? __m Culvert: Within Reach: Yes or No *Upstream: Yes or No if yes, how far? __m Pipes: Within Reach: Yes or No * Upstream: Yes or No if yes, how far? __m *Distance of site from tributary mouth/main river channel: ______ km
Riparian vegetation (within 18 meters)	Width of vegetated riparian zone (looking downstream): Left bank_________ m Right bank________m (estimated or measured?) Indicate the dominant type and record the dominant species present (circle one): Trees Shrubs Grasses Herbaceous None Dominant species (if known):
Large woody debris	Abundance of LWD (# logs ≥ 10 cm diameter in stream reach): Length of reach measured:__________m
Aquatic vegetation	Indicate the dominant type and record the dominant species present (circle one): Rooted emergent rooted submergent floating algae attached algae rooted floating free floating Portion of the reach with aquatic vegetation: _____ %
Sediment substrate	Odors (circle one): Normal Sewage Petroleum Chemical Sulfur None Other: Oils (circle one): Absent Slight Moderate Profuse
Water Quality in Channel	**Circle all that apply:** Debris Obvious Pollution: Sludge, Sawdust, Paper Fiber, Sand, Silt, Sewage, Oily Sheen, Trash, Iron, Scum, None Water Clarity**:** Clear, Slightly Turbid, Moderately Turbid, Very Turbid Water Color**:** Clear, Green, Milky, Brown, Tannic (L M H), Gray, Metallic, Reddish Odors**:** None, Musty, Fishy, Sewage, Manure, Sulfur(eggs), Oily/gas

Local Land Use (within about ¼ mile of site; adjacent and upstream)
Check “1” if present, “2” if clearly having an impact on a stream.

0	**1**	**2**	**Residential**
0	0	0	Single-family housing
0	0	0	Multi-family housing
0	0	0	Lawns
0	0	0	Commercial/Institutional

0	**1**	**2**	**Roads, etc.**
0	0	0	Paved roads or bridges
0	0	0	Unpaved roads

0	**1**	**2**	**Construction underway on:**
0	0	0	Housing development
0	0	0	Commercial development
0	0	0	Road bridge construction/repair

0	**1**	**2**	**Agricultural**
0	0	0	Grazing Land
0	0	0	Feeding lots or animal holding areas
0	0	0	Cropland
0	0	0	Inactive agricultural land/fields

0	**1**	**2**	**Recreation**
0	0	0	Power boating
0	0	0	Golfing
0	0	0	Camping
0	0	0	Swimming/fishing/canoeing
0	0	0	Hiking/paths

0	**1**	**2**	**Other**
0	0	0	Mining or gravel pits
0	0	0	Logging
0	0	0	Industry
0	0	0	Oil and gas drilling
0	0	0	Trash dump
0	0	0	Landfill

Comments:

Habitat Assessment Data Sheet (2011-2012)

STREAM NAME:	SITE CODE:
DATE/TIME:	INVESTIGATORS:
LATITUDE	LONGITUDE:
SITE DESCRIPTION:	WEATHER CONDITIONS:

Habitat Parameter	Reference	Good	Fair	Poor
Epifaunal Substrate/Cover	Greater than 70% (50% for low gradient streams) of stream bed and lower banks covered with mix of substrates favorable for epifaunal colonization and fish cover; substrates include snags, submerged logs, undercut banks, and unembedded cobbles and boulders (for high gradient)	40-70% (30-50% for low gradient streams) of stream bed and lower banks covered with a mix of substrates favorable for epifaunal colonization and fish cover	20-40% (10-30% for low gradient streams) of stream bed and lower banks covered with substrates favorable for epifaunal colonization and fish cover; few substrate types present	Less than 20% (10% for low gradient streams) of stream bed and lower banks covered with substrates favorable for epifaunal colonization and fish cover; few substrate types present
	20 19 18 17 16	**15 14 13 12 11**	**10 9 8 7 6**	**5 4 3 2 1**
Embeddedness (high gradient)	Gravel, cobble, and boulder particles are 0-25% surrounded by fine sediment. Layering of cobble provides diversity of niche space.	Gravel, cobble, and boulder particles are 25-50% surrounded by fine sediment.	Gravel, cobble, and boulder particles are 50-75% surrounded by fine sediment. Little open space between particles.	Gravel, cobble, and boulder particles are more than 75% surrounded by fine sediment. Almost no open space between particles.
	20 19 18 17 16	**15 14 13 12 11**	**10 9 8 7 6**	**5 4 3 2 1**
Pool Substrate Characterization (low gradient)	Characterization (low gradient) Mixture of substrate materials, with gravel and firm sand prevalent; root mats and submerged vegetation common.	Mixture of soft sand, mud, or clay; mud may be dominant; some root mats and submerged vegetation present.	All mud or clay or sand bottom; little or no root mat; no submerged vegetation.	Hard-pan clay or bedrock; no root mat or vegetation.
	20 19 18 17 16	**15 14 13 12 11**	**10 9 8 7 6**	**5 4 3 2 1**
Velocity/Depth Patterns (high gradient)	All 4 velocity/depth patterns present: slow-deep, slow-shallow, fast-deep, fast-shallow. Slow is < 1 ft/s. (0.3 m/s), deep is > 1.5 ft (0.5 m).	Only 3 of the 4 patterns present (if fast-shallow is missing, score lower than if missing other regimes.	Only 2 of the 4 patterns present (if fast-shallow or slow-shallow are missing, score low).	Dominated by 1 velocity/ depth pattern (usually slow-deep).
	20 19 18 17 16	**15 14 13 12 11**	**10 9 8 7 6**	**5 4 3 2 1**
Pool Variability (low gradient)	Even mix of large-shallow, large-deep, small-shallow, small-deep pools present.	Majority of pools large-deep; very few shallow.	Shallow pools much more prevalent than deep pools.	Majority of pools small-shallow or pools absent.
	20 19 18 17 16	**15 14 13 12 11**	**10 9 8 7 6**	**5 4 3 2 1**

Sediment Deposition	Little or no enlargement of mid-channel bars or point bars and < 5% (20% in low gradient streams) of the bottom affected by sediment deposition.	Some new increase in bar formation, mostly from gravel, sand or fine sediment; -30% (20-50% in low gradient streams) of the bottom affected; slight deposition in pools.	Moderate deposition of new gravel, sand or fine sediment on old and new bars; 30-50% (50-80% in low gradient streams) of the bottom affected; sediment deposits at obstructions, constrictions, and bends; moderate deposition of pools prevalent.	Heavy deposits of fine material, increased bar development; > 50% (80% in low gradient streams) of the bottom changing frequently; pools almost absent due to substantial sediment deposition.
	20 19 18 17 16	**15 14 13 12 11**	**10 9 8 7 6**	**5 4 3 2 1**
Channel Flow Status	Water reaches base of both lower banks, and <10% of channel bed substrate is exposed.	Water fills >75% of the available channel; or <25% of channel bed substrate is exposed.	Water fills 25-75% of the available channel, and/or riffle substrates are mostly exposed.	Very little water in channel and mostly present as standing pools.
	20 19 18 17 16	**15 14 13 12 11**	**10 9 8 7 6**	**5 4 3 2 1**
Channel Alteration	Channelization in the form of dredging, straightening, berms or streambank armoring absent; stream with natural pattern.	Some channel alterations present along 10-20% of segment, usually in areas of bridge abutments; evidence of past channelization, (greater than past 20 yr) may be present, but recent channelization is not present.	Channelization along 20-80% of stream segment ; riprap or armoring present on both banks.	Over 80% of the stream segment channelized and disrupted. Instream habitat greatly altered or removed entirely.
	20 19 18 17 16	**15 14 13 12 11**	**10 9 8 7 6**	**5 4 3 2 1**
Frequency of riffles/steps (high gradient)	Occurrence of riffles/steps relatively frequent; ratio of distance between riffles is 5-7 times (steps 3-5 times) stream width; variety of habitat is key. In streams where riffles/steps are continuous, presence of boulders or other large, natural obstruction is important.	Occurrence of riffles/steps infrequent; distance between riffles is 7-15 times (steps 5-15 times) stream width.	Occasional riffle/step or bend; bottom contours provide some habitat; distance between riffles/steps is 15 to 25 stream widths.	Generally all flat water or shallow riffles/steps; poor habitat; distance between riffles/steps is >25 stream widths. Mostly runs.
	20 19 18 17 16	**15 14 13 12 11**	**10 9 8 7 6**	**5 4 3 2 1**
Channel Sinuosity (low gradient)	The bends in the stream increase the stream length 2.5 to 4 times longer than the straight down-valley length.	The bends in the stream increase the stream length 1.5 to 2.5 times longer than the straight down-valley length.	The bends in the stream increase the stream length 1 to 1.5 times longer than the straight down-valley length.	Channel straight; waterway has been channelized for a long distance.
	20 19 18 17 16	**15 14 13 12 11**	**10 9 8 7 6**	**5 4 3 2 1**

Bank Stability (score each bank) *Note: determine left or right side by facing downstream.*	Banks stable; evidence of erosion or bank failure absent or minimal; < 5% of bank affected.	Moderately stable; infrequent, small areas of erosion mostly re-vegetated. 5-30% of bank in segment (or reach) has areas of erosion.	Moderately unstable; 30-60% of bank in segment (or reach) has areas of erosion; high erosion potential from crumbling, unvegetated banks during floods.	Unstable; many eroded areas; "raw" areas frequent along straight sections and bends; obvious bank sloughing; 60-100% of bank has erosional scars.
Score _____ (LB)	**Left bank 10 9**	**8 7 6**	**5 4 3**	**2 1 0**
Score _____ (RB)	**Right bank 10 9**	**8 7 6**	**5 4 3**	**2 1 0**
Bank Vegetative (score each bank) *Note: determine left or right side by facing downstream.*	More than 90% of the streambank surfaces and immediate riparian zone covered by native vegetation, including trees, understory shrubs, or herbaceous vegetation; vegetative disruption through grazing or mowing minimal or not evident; almost all plants allowed to grow naturally.	70-90% of the streambank surfaces covered by native vegetation, but one class of plants is not well-represented; disruption evident but not affecting full plant growth potential to any great extent; more than one-half of the potential plant stubble height remaining.	50-70% of the streambank surfaces covered by vegetation; disruption obvious; patches of bare soil or closely cropped vegetation common; less than one-half of the potential plant stubble height remaining.	Less than 50% of the streambank surfaces covered by vegetation; disruption of streambank vegetation is very high; vegetation has been removed to 5 centimeters or less in average stubble height.
Score _____ (LB)	**Left bank 10 9**	**8 7 6**	**5 4 3**	**2 1 0**
Score _____ (RB)	**Right bank 10 9**	**8 7 6**	**5 4 3**	**2 1 0**
Riparian Vegetative Zone Width (score each side of channel. *Note: determine left or right side by facing downstream*)	Width of naturally vegetated riparian zone >100 feet; human activities, (i.e., parking lots, roadbeds, clear-cuts, lawns, or crops) and grazing have not impacted zone.	Width of riparian zone 50 - 100 ft; human activities and grazing have impacted zone only minimally.	Width of riparian zone 25 - 50 ft.; human activities and grazing have impacted zone a great deal.	Width of riparian zone < 25 feet: little or no riparian vegetation due to human activities.
Score _____ (LB)	**Left bank 10 9**	**8 7 6**	**5 4 3**	**2 1 0**
Score _____ (RB)	**Right bank 10 9**	**8 7 6**	**5 4 3**	**2 1 0**
HABITAT SCORE	**Sum of score for all 10 categories for stream type (high or low gradient): _______ x 100 =** **200**			

Comments:

5 HABITAT ASSESSMENT AND PHYSICOCHEMICAL PARAMETERS

An evaluation of habitat quality is critical to any assessment of ecological integrity and should be performed at each site at the time of the biological sampling. In general, habitat and biological diversity in rivers are closely linked (Raven et al. 1998). In the truest sense, "habitat" incorporates all aspects of physical and chemical constituents along with the biotic interactions. In these protocols, the definition of "habitat" is narrowed to the quality of the instream and riparian habitat that influences the structure and function of the aquatic community in a stream. The presence of an altered habitat structure is considered one of the major stressors of aquatic systems (Karr et al. 1986). The presence of a degraded habitat can sometimes obscure investigations on the effects of toxicity and/or pollution. The assessments performed by many water resource agencies include a general description of the site, a physical characterization and water quality assessment, and a visual assessment of instream and riparian habitat quality. Some states (e.g., Idaho DEQ and Illinois EPA) include quantitative measurements of physical parameters in their habitat assessment. Together these data provide an integrated picture of several of the factors influencing the biological condition of a stream system. These assessments are not as comprehensive as needed to adequately identify all causes of impact. However, additional investigation into hydrological modification of water courses and drainage patterns can be conducted, once impairment is noted.

The habitat quality evaluation can be accomplished by characterizing selected physicochemical parameters in conjunction with a systematic assessment of physical structure. Through this approach, key features can be rated or scored to provide a useful assessment of habitat quality.

5.1 PHYSICAL CHARACTERISTICS AND WATER QUALITY

Both physical characteristics and water quality parameters are pertinent to characterization of the stream habitat. An example of the data sheet used to characterize the physical characteristics and water quality of a site is shown in Appendix A. The information required includes measurements of physical characterization and water quality made routinely to supplement biological surveys.

Physical characterization includes documentation of general land use, description of the stream origin and type, summary of the riparian vegetation features, and measurements of instream parameters such as width, depth, flow, and substrate. The water quality discussed in these protocols are *in situ* measurements of standard parameters that can be taken with a water quality instrument. These are generally instantaneous measurements taken at the time of the survey. Measurements of certain parameters, such as temperature, dissolved oxygen, and turbidity, can be taken over a diurnal cycle and will require instrumentation that can be left in place for extended periods or collects water samples at periodic intervals for measurement. In addition, water samples may be desired to be collected for selected chemical analysis. These chemical samples are transported to an analytical laboratory for processing. The combination of this information (physical characterization and water quality) will provide insight as to the ability of the stream to support a healthy aquatic community, and to the presence of chemical and non-chemical stressors to the stream ecosystem. Information requested in this section (Appendix A-1, Form 1) is standard

to many aquatic studies and allows for some comparison among sites. Additionally, conditions that may significantly affect aquatic biota are documented.

5.1.1 Header Information (Station Identifier)

The header information is identical on all data sheets and requires sufficient information to identify the station and location where the survey was conducted, date and time of survey, and the investigators responsible for the quality and integrity of the data. The stream name and river basin identify the watershed and tributary; the location of the station is described in the narrative to help identify access to the station for repeat visits. The rivermile (if applicable) and latitude/longitude are specific locational data for the station. The station number is a code assigned by the agency that will associate the sample and survey data with the station. The STORET number is assigned to each datapoint for inclusion in USEPA's STORET system. The stream class is a designation of the grouping of homogeneous characteristics from which assessments will be made. For instance, Ohio EPA uses ecoregions and size of stream, Florida DEP uses bioregions (aggregations of subecoregions), and Arizona DEQ uses elevation as a means to identify stream classes. Listing the agency and investigators assigns responsibility to the data collected from the station at a specific date and time. The reason for the survey is sometimes useful to an agency that conducts surveys for various programs and purposes.

5.1.2 Weather Conditions

Note the present weather conditions on the day of the survey and those immediately preceding the day of the survey. This information is important to interpret the effects of storm events on the sampling effort.

5.1.3 Site Location/Map

To complete this phase of the bioassessment, a photograph may be helpful in identifying station location and documenting habitat conditions. Any observations or data not requested but deemed important by the field observer should be recorded. A hand-drawn map is useful to illustrate major landmarks or features of the channel morphology or orientation, vegetative zones, buildings, etc. that might be used to aid in data interpretation.

5.1.4 Stream Characterization

Stream Subsystem: In regions where the perennial nature of streams is important, or where the tidal influence of streams will alter the structure and function of communities, this parameter should be noted.

Stream Type: Communities inhabiting coldwater streams are markedly different from those in warmwater streams, many states have established temperature criteria that differentiate these 2 stream types.

Stream Origin: Note the origination of the stream under study, if it is known. Examples are glacial, montane, swamp, and bog. As the size of the stream or river increases, a mixture of origins of tributaries is likely.

5.1.5 Watershed Features

Collecting this information usually requires some effort initially for a station. However, subsequent surveys will most likely not require an in-depth research of this information.

Predominant Surrounding Land Use Type: Document the prevalent land-use type in the catchment of the station (noting any other land uses in the area which, although not predominant, may potentially affect water quality). Land use maps should be consulted to accurately document this information.

Local Watershed Nonpoint Source Pollution: This item refers to problems and potential problems in the watershed. Nonpoint source pollution is defined as diffuse agricultural and urban runoff. Other compromising factors in a watershed that may affect water quality include feedlots, constructed wetlands, septic systems, dams and impoundments, mine seepage, etc.

Local Watershed Erosion: The existing or potential detachment of soil within the local watershed (the portion of the watershed or catchment that directly affects the stream reach or station under study) and its movement into the stream is noted. Erosion can be rated through visual observation of watershed and stream characteristics (note any turbidity observed during water quality assessment below).

5.1.6 Riparian Vegetation

An acceptable riparian zone includes a buffer strip of a minimum of 18 m (Barton et al. 1985) from the stream on either side. The acceptable width of the riparian zone may also be variable depending on the size of the stream. Streams over 4 m in width may require larger riparian zones. The vegetation within the riparian zone is documented here as the dominant type and species, if known.

5.1.7 Instream Features

Instream features are measured or evaluated in the sampling reach and catchment as appropriate.

Estimated Reach Length: Measure or estimate the length of the sampling reach. This information is important if reaches of variable length are surveyed and assessed.

Estimated Stream Width (in meters, m): Estimate the distance from bank to bank at a transect representative of the stream width in the reach. If variable widths, use an average to find that which is representative for the given reach.

Sampling Reach Area (m^2): Multiply the sampling reach length by the stream width to obtain a calculated surface area.

Estimated Stream Depth (m): Estimate the vertical distance from water surface to stream bottom at a representative depth (use instream habitat feature that is most common in reach) to obtain average depth.

Velocity: Measure the surface velocity in the thalweg of a representative run area. If measurement is not done, estimate the velocity as slow, moderate, or fast.

Canopy Cover: Note the general proportion of open to shaded area which best describes the amount of cover at the sampling reach or station. A densiometer may be used in place of visual estimation.

High Water Mark (m): Estimate the vertical distance from the bankfull margin of the stream bank to the peak overflow level, as indicated by debris hanging in riparian or floodplain vegetation, and deposition of silt or soil. In instances where bank overflow is rare, a high water mark may not be evident.

Proportion of Reach Represented by Stream Morphological Types: The proportion represented by riffles, runs, and pools should be noted to describe the morphological heterogeneity of the reach.

Channelized: Indicate whether or not the area around the sampling reach or station is channelized (e.g., straightening of stream, bridge abutments and road crossings, diversions, etc.).

Dam Present: Indicate the presence or absence of a dam upstream in the catchment or downstream of the sampling reach or station. If a dam is present, include specific information relating to alteration of flow.

5.1.8 Large Woody Debris

Large Woody Debris (LWD) density, defined and measured as described below, has been used in regional surveys (Shields et al. 1995) and intensive studies of degraded and restored streams (Shields et al. 1998). The method was developed for sand or sand-and-gravel bed streams in the Southeastern U.S. that are wadeable at baseflow, with water widths between 1 and 30 m (Cooper and Testa 1999).

Cooper and Testa's (1999) procedure involves measurements based on visual estimates taken by a wading observer. Only woody debris actually in contact with stream water is counted. Each woody debris formation with a surface area in the plane of the water surface >0.25 m^2 is recorded. The estimated length and width of each formation is recorded on a form or marked directly onto a stream reach drawing. Estimates are made to the nearest 0.5 m , and formations with length or width less than 0.5 m are not counted. Recorded length is maximum width in the direction perpendicular to the length. Maximum actual length and width of a limb, log, or accumulation are not considered.

If only a portion of the log/limb is in contact with the water, only that portion in contact is measured. Root wads and logs/limbs in the water margin are counted if they contact the water, and are arbitrarily given a width of 0.5 m Lone individual limbs and logs are included in the determination if their diameter is 10 cm or larger (Keller and Swanson 1979, Ward and Aumen 1986). Accumulations of smaller limbs and logs are included if the formation total length or width is 0.5 m or larger. Standing trees and stumps within the stream are also recorded if their length and width exceed 0.5 m.

The length and width of each LWD formation are then multiplied, and the resulting products are summed to give the aquatic habitat area directly influenced. This area is then divided by the water

surface area (km^2) within the sampled reach (obtained by multiplying the average water surface width by reach length) to obtain LWD density. Density values of 10^3 to 10^4 m^2/km^2 have been reported for channelized and incised streams and on the order of 10^5 m^2/km^2 for non-incised streams (Shields et al. 1995 and 1998). This density is not an expression of the volume of LWD, but rather a measure of LWD influence on velocity, depth, and cover.

5.1.9 Aquatic Vegetation

The general type and relative dominance of aquatic plants are documented in this section. Only an estimation of the extent of aquatic vegetation is made. Besides being an ecological assemblage that responds to perturbation, aquatic vegetation provides refugia and food for aquatic fauna. List the species of aquatic vegetation, if known.

5.1.10 Water Quality

Temperature (°C), Conductivity or "Specific Conductance" (µohms), Dissolved Oxygen (µg/L), pH, Turbidity: Measure and record values for each of the water quality parameters indicated, using the appropriate calibrated water quality instrument(s). Note the type of instrument and unit number used.

Water Odors: Note those odors described (or include any other odors not listed) that are associated with the water in the sampling area.

Water Surface Oils: Note the term that best describes the relative amount of any oils present on the water surface.

Turbidity: If turbidity is not measured directly, note the term which, based upon visual observation, best describes the amount of material suspended in the water column.

5.1.11 Sediment/Substrate

Sediment Odors: Disturb sediment in pool or other depositional areas and note any odors described (or include any other odors not listed) which are associated with sediment in the sampling reach.

Sediment Oils: Note the term which best describes the relative amount of any sediment oils observed in the sampling area.

Sediment Deposits: Note those deposits described (or include any other deposits not listed) that are present in the sampling reach. Also indicate whether the undersides of rocks not deeply embedded are black (which generally indicates low dissolved oxygen or anaerobic conditions).

Inorganic Substrate Components: Visually estimate the relative proportion of each of the 7 substrate/particle types listed that are present over the sampling reach.

Organic Substrate Components: Indicate relative abundance of each of the 3 substrate types listed.

5.2 A VISUAL-BASED HABITAT ASSESSMENT

Biological potential is limited by the quality of the physical habitat, forming the template within which biological communities develop (Southwood 1977). Thus, habitat assessment is defined as the evaluation of the structure of the surrounding physical habitat that influences the quality of the water resource and the condition of the resident aquatic community (Barbour et al. 1996a). For streams, an encompassing approach to assessing structure of the habitat includes an evaluation of the variety and quality of the substrate, channel morphology, bank structure, and riparian vegetation. Habitat parameters pertinent to the assessment of habitat quality include those that characterize the stream "micro scale" habitat (e.g., estimation of embeddeddness), the "macro scale" features (e.g., channel morphology), and the riparian and bank structure features that are most often influential in affecting the other parameters.

Rosgen (1985, 1994) presented a stream and river classification system that is founded on the premise that dynamically-stable stream channels have a morphology that provides appropriate distribution of flow energy during storm events. Further, he identifies 8 major variables that affect the stability of channel morphology, but are not mutually independent: channel width, channel depth, flow velocity, discharge, channel slope, roughness of channel materials, sediment load and sediment particle size distribution. When streams have one of these characteristics altered, some of their capability to dissipate energy properly is lost (Leopold et al. 1964, Rosgen 1985) and will result in accelerated rates of channel erosion. Some of the habitat structural components that function to dissipate flow energy are:

EQUIPMENT/SUPPLIES NEEDED FOR HABITAT ASSESSMENT AND PHYSICAL/WATER QUALITY CHARACTERIZATION

- Physical Characterization and Water Quality Field Data Sheet*
- Habitat Assessment Field Data Sheet*
- clipboard
- pencils or waterproof pens
- 35 mm camera (may be digital)
- video camera (optional)
- upstream/downstream “arrows” or signs for photographing and documenting sampling reaches
- Flow or velocity meter
- *In situ* water quality meters
- Global Positioning System (GPS) Unit

* It is helpful to copy field sheets onto water-resistant paper for use in wet weather conditions

! sinuosity

! roughness of bed and bank materials

! presence of point bars (slope is an important characteristic)

! vegetative conditions of stream banks and the riparian zone

! condition of the floodplain (accessibility from bank, overflow, and size are important characteristics).

Measurement of these parameters or characteristics serve to stratify and place streams into distinct classifications. However, none of these habitat classification techniques attempt to differentiate the quality of the habitat and the ability of the habitat to support the optimal biological condition of the

region. Much of our understanding of habitat relationships in streams has emerged from comparative studies that describe statistical relationships between habitat variables and abundance of biota (Hawkins et al. 1993). However, in response to the need to incorporate broader scale habitat assessments in water resource programs, 2 types of approaches for evaluating habitat structure have been developed. In the first, the Environmental Monitoring and Assessment Program (EMAP) of the USEPA and the National Water-Quality Assessment Program (NAWQA) of the USGS developed techniques that incorporate measurements of various features of the instream, channel, and bank morphology (Meader et al. 1993, Klemm and Lazorchak 1994). These techniques provide a relatively comprehensive characterization of the physical structure of the stream sampling reach and its surrounding floodplain. The second type was a more rapid and qualitative habitat assessment approach that was developed to describe the overall quality of the physical habitat (Ball 1982, Ohio EPA 1987, Plafkin et al. 1989, Barbour and Stribling 1991, 1994, Rankin 1991, 1995). In this document, the more rapid visual-based approach is described. A cursory overview of the more quantitative approaches to characterizing the physical structure of the habitat is provided.

The habitat assessment matrix developed for the Rapid Bioassessment Protocols (RBPs) in Plafkin et al. (1989) were originally based on the Stream Classification Guidelines for Wisconsin developed by Ball (1982) and "*Methods of Evaluating Stream, Riparian, and Biotic Conditions*" developed by Platts et al. (1983). Barbour and Stribling (1991, 1994) modified the habitat assessment approach originally developed for the RBPs to include additional assessment parameters for high gradient streams and a more appropriate parameter set for low gradient streams (Appendix A-1, Forms 2,3). All parameters are evaluated and rated on a numerical scale of 0 to 20 (highest) for each sampling reach. The ratings are then totaled and compared to a reference condition to provide a final habitat ranking. Scores increase as habitat quality increases. To ensure consistency in the evaluation procedure, descriptions of the physical parameters and relative criteria are included in the rating form.

The Environmental Agency of Great Britain (Environment Agency of England and Wales, Scottish Environment Protection Agency, and Environment and Heritage Service of Northern Ireland) have developed a River Habitat Survey (RHS) for characterizing the quality of their streams and rivers (Raven et al. 1998). The approach used in Great Britain is similar to the visual-based habitat assessment used in the US in that scores are assigned to ranges of conditions of various habitat parameters.

A biologist who is well versed in the ecology and zoogeography of the region can generally recognize optimal habitat structure as it relates to the biological community. The ability to accurately assess the quality of the physical habitat structure using a visual-based approach depends on several factors:

- ! the parameters selected to represent the various features of habitat structure need to be relevant and clearly defined

- ! a continuum of conditions for each parameter must exist that can be characterized from the optimum for the region or stream type under study to the poorest situation reflecting substantial alteration due to anthropogenic activities

- ! the judgement criteria for the attributes of each parameter should minimize subjectivity through either quantitative measurements or specific categorical choices

- ! the investigators are experienced in or adequately trained for stream assessments in the region under study (Hannaford et al. 1997)

- ! adequate documentation and ongoing training is maintained to evaluate and correct errors resulting in outliers and aberrant assessments.

Habitat evaluations are first made on instream habitat, followed by channel morphology, bank structural features, and riparian vegetation. Generally, a single, comprehensive assessment is made that incorporates features of the entire sampling reach as well as selected features of the catchment. Additional assessments may be made on neighboring reaches to provide a broader evaluation of habitat quality for the stream ecosystem. The actual habitat assessment process involves rating the 10 parameters as optimal, suboptimal, marginal, or poor based on the criteria included on the Habitat Assessment Field Data Sheets (Appendix A-1, Forms 2,3). Some state programs, such as Florida Department of Environmental Protection (DEP) (1996) and Mid-Atlantic Coastal Streams Workgroup (MACS) (1996) have adapted this approach using somewhat fewer and different parameters.

Reference conditions are used to scale the assessment to the "best attainable" situation. This approach is critical to the assessment because stream characteristics will vary dramatically across different regions (Barbour and Stribling 1991). The ratio between the score for the test station and the score for the reference condition provides a percent comparability measure for each station. The station of interest is then classified on the basis of its similarity to expected conditions (reference condition), and its apparent potential to support an acceptable level of biological health. Use of a percent comparability evaluation allows for regional and stream-size differences which affect flow or velocity, substrate, and channel morphology. Some regions are characterized by streams having a low channel gradient, such as coastal plains or prairie regions.

Other habitat assessment approaches or a more rigorously quantitative approach to measuring the habitat parameters may be used (See Klemm and Lazorchak 1994, Kaufmann and Robison 1997, Meader et al. 1993). However, holistic and rapid assessment of a wide variety of habitat attributes along with other types of data is critical if physical measurements are to be used to best advantage in interpreting biological data. A more detailed discussion of the relationship between habitat quality and biological condition is presented in Chapter 10.

A generic habitat assessment approach based on visual observation can be separated into 2 basic approaches—one designed for high-gradient streams and one designed for low-gradient streams. High-gradient or riffle/run prevalent streams are those in moderate to high gradient landscapes. Natural high-gradient streams have substrates primarily composed of coarse sediment particles (i.e., gravel or larger) or frequent coarse particulate aggregations along stream reaches. Low-gradient or glide/pool prevalent streams are those in low to moderate gradient landscapes. Natural low-gradient streams have substrates of fine sediment or infrequent aggregations of more coarse (gravel or larger) sediment particles along stream reaches. The entire sampling reach is evaluated for each parameter. Descriptions of each parameter and its relevance to instream biota are presented in the following discussion. Parameters that are used only for high-gradient prevalent streams are marked with an “a”; those for low-gradient dominant streams, a “b”. If a parameter is used for both stream types, it is not marked with a letter. A brief set of decision criteria is given

for each parameter corresponding to each of the 4 categories reflecting a continuum of conditions on the field sheet (optimal, suboptimal, marginal, and poor). Refer to Appendix A-1, Forms 2 and 3, for a complete field assessment guide.

Procedure for Performing Habitat Assessment

1. Select the reach to be assessed. The habitat assessment is performed on the same 100 m reach (or other reach designation [e.g., 40 x stream wetted width]) from which the biological sampling is conducted. Some parameters require an observation of a broader section of the catchment than just the sampling reach.

2. Complete the station identification section of each field data sheet and habitat assessment form.

3. It is best for the investigators to obtain a close look at the habitat features to make an adequate assessment. If the physical and water quality characterization and habitat assessment are done before the biological sampling, care must be taken to avoid disturbing the sampling habitat.

4. Complete the **Physical Characterization and Water Quality Field Data Sheet**. Sketch a map of the sampling reach on the back of this form.

5. Complete the **Habitat Assessment Field Data Sheet**, in a team of 2 or more biologists, if possible, to come to a consensus on determination of quality. Those parameters to be evaluated on a scale greater than a sampling reach require traversing the stream corridor to the extent deemed necessary to assess the habitat feature. As a general rule-of-thumb, use 2 lengths of the sampling reach to assess these parameters.

Quality Assurance Procedures

1. Each biologist is to be trained in the visual-based habitat assessment technique for the applicable region or state.

2. The judgment criteria for each habitat parameter are calibrated for the stream classes under study. Some text modifications may be needed on a regional basis.

3. Periodic checks of assessment results are completed using pictures of the sampling reach and discussions among the biologists in the agency.

Parameters to be evaluated in sampling reach:

1 EPIFAUNAL SUBSTRATE/AVAILABLE COVER

high and low gradient streams

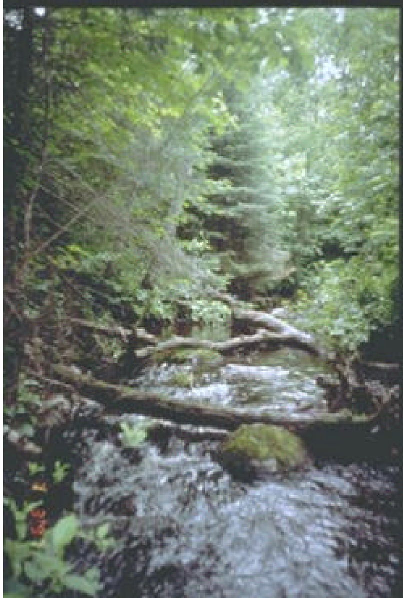
Includes the relative quantity and variety of natural structures in the stream, such as cobble (riffles), large rocks, fallen trees, logs and branches, and undercut banks, available as refugia, feeding, or sites for spawning and nursery functions of aquatic macrofauna. A wide variety and/or abundance of submerged structures in the stream provides macroinvertebrates and fish with a large number of niches, thus increasing habitat diversity. As variety and abundance of cover decreases, habitat structure becomes monotonous, diversity decreases, and the potential for recovery following disturbance decreases. Riffles and runs are critical for maintaining a variety and abundance of insects in most high-gradient streams and serving as spawning and feeding refugia for certain fish. The extent and quality of the riffle is an important factor in the support of a healthy biological condition in high-gradient streams. Riffles and runs offer a diversity of habitat through variety of particle size, and, in many small high-gradient streams, will provide the most stable habitat. Snags and submerged logs are among the most productive habitat structure for macroinvertebrate colonization and fish refugia in low-gradient streams. However, "new fall" will not yet be suitable for colonization.

Selected References

Wesche et al. 1985, Pearsons et al. 1992, Gorman 1988, Rankin 1991, Barbour and Stribling 1991, Plafkin et al. 1989, Platts et al. 1983, Osborne et al. 1991, Benke et al. 1984, Wallace et al. 1996, Ball 1982, MacDonald et al. 1991, Reice 1980, Clements 1987, Hawkins et al. 1982, Beechie and Sibley 1997.

Habitat Parameter	**Condition Category**			
	Optimal	**Suboptimal**	**Marginal**	**Poor**
1. Epifaunal Substrate/ Available Cover **(high and low gradient)**	Greater than 70% (50% for low gradient streams) of substrate favorable for epifaunal colonization and fish cover; mix of snags, submerged logs, undercut banks, cobble or other stable habitat and at stage to allow full colonization potential (i.e., logs/snags that are not new fall and not transient).	40-70% (30-50% for low gradient streams) mix of stable habitat; well-suited for full colonization potential; adequate habitat for maintenance of populations; presence of additional substrate in the form of newfall, but not yet prepared for colonization (may rate at high end of scale).	20-40% (10-30% for low gradient streams) mix of stable habitat; habitat availability less than desirable; substrate frequently disturbed or removed.	Less than 20% (10% for low gradient streams) stable habitat; lack of habitat is obvious; substrate unstable or lacking.
SCORE	20 19 18 17 16	15 14 13 12 11	10 9 8 7 6	5 4 3 2 1 0

1a. Epifaunal Substrate/Available Cover—High Gradient



Optimal Range

Poor Range

1b. Epifaunal Substrate/Available Cover—Low Gradient

Optimal Range (*Mary Kay Corazalla, U. of Minn.*)

Poor Range

2a EMBEDDEDNESS

high gradient streams

Refers to the extent to which rocks (gravel, cobble, and boulders) and snags are covered or sunken into the silt, sand, or mud of the stream bottom. Generally, as rocks become embedded, the surface area available to macroinvertebrates and fish (shelter, spawning, and egg incubation) is decreased. Embeddedness is a result of large-scale sediment movement and deposition, and is a parameter evaluated in the riffles and runs of high-gradient streams. The rating of this parameter may be variable depending on where the observations are taken. To avoid confusion with sediment deposition (another habitat parameter), observations of embeddedness should be taken in the upstream and central portions of riffles and cobble substrate areas.

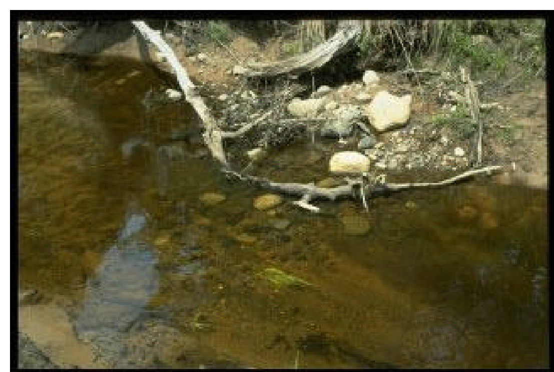
Selected References

Ball 1982, Osborne et al. 1991, Barbour and Stribling 1991, Platts et al. 1983, MacDonald et al. 1991, Rankin 1991, Reice 1980, Clements 1987, Benke et al. 1984, Hawkins et al. 1982, Burton and Harvey 1990.

Habitat Parameter	Condition Category			
	Optimal	**Suboptimal**	**Marginal**	**Poor**
2.a Embeddedness **(high gradient)**	Gravel, cobble, and boulder particles are 0-25% surrounded by fine sediment. Layering of cobble provides diversity of niche space.	Gravel, cobble, and boulder particles are 25-50% surrounded by fine sediment.	Gravel, cobble, and boulder particles are 50-75% surrounded by fine sediment.	Gravel, cobble, and boulder particles are more than 75% surrounded by fine sediment.
SCORE	20 19 18 17 16	15 14 13 12 11	10 9 8 7 6	5 4 3 2 1 0

2a. Embeddedness—High Gradient

Optimal Range *(William Taft, MI DNR)*



Poor Range *(William Taft, MI DNR)*

2b POOL SUBSTRATE CHARACTERIZATION

low gradient streams

Evaluates the type and condition of bottom substrates found in pools. Firmer sediment types (e.g., gravel, sand) and rooted aquatic plants support a wider variety of organisms than a pool substrate dominated by mud or bedrock and no plants. In addition, a stream that has a uniform substrate in its pools will support far fewer types of organisms than a stream that has a variety of substrate types.

Selected References

Beschta and Platts 1986, U.S. EPA 1983.

Habitat Parameter	Condition Category			
	Optimal	**Suboptimal**	**Marginal**	**Poor**
2b. Pool Substrate Characterization **(low gradient)**	Mixture of substrate materials, with gravel and firm sand prevalent; root mats and submerged vegetation common.	Mixture of soft sand, mud, or clay; mud may be dominant; some root mats and submerged vegetation present.	All mud or clay or sand bottom; little or no root mat; no submerged vegetation.	Hard-pan clay or bedrock; no root mat or submerged vegetation.
SCORE	20 19 18 17 16	15 14 13 12 11	10 9 8 7 6	5 4 3 2 1 0

2b. Pool Substrate Characterization—Low Gradient

Optimal Range
(Mary Kay Corazalla, U. of Minn.)

Poor Range

3a VELOCITY/DEPTH COMBINATIONS

high gradient streams

Patterns of velocity and depth are included for high-gradient streams under this parameter as an important feature of habitat diversity. The best streams in most high-gradient regions will have all 4 patterns present: (1) slow-deep, (2) slow-shallow, (3) fast-deep, and (4) fast-shallow. The general guidelines are 0.5 m depth to separate shallow from deep, and 0.3 m/sec to separate fast from slow. The occurrence of these 4 patterns relates to the stream's ability to provide and maintain a stable aquatic environment.

Selected References

Ball 1982, Brown and Brussock 1991, Gore and Judy 1981, Oswood and Barber 1982.

Habitat Parameter	Condition Category			
	Optimal	**Suboptimal**	**Marginal**	**Poor**
3a. Velocity/ Depth Regimes **(high gradient)**	All 4 velocity/depth regimes present (slow-deep, slow-shallow, fast-deep, fast-shallow). (slow is <0.3 m/s, deep is >0.5 m)	Only 3 of the 4 regimes present (if fast-shallow is missing, score lower than if missing other regimes).	Only 2 of the 4 habitat regimes present (if fast-shallow or slow-shallow are missing, score low).	Dominated by 1 velocity/ depth regime (usually slow-deep).
SCORE	20 19 18 17 16	15 14 13 12 11	10 9 8 7 6	5 4 3 2 1 0

3a. Velocity/Depth Regimes—High Gradient

Optimal Range *(Mary Kay Corazalla, U. of Minn.)*
(arrows emphasize different velocity/depth regimes)

Poor Range *(William Taft, MI DNR)*

3b POOL VARIABILITY

low gradient streams

Rates the overall mixture of pool types found in streams, according to size and depth. The 4 basic types of pools are large-shallow, large-deep, small-shallow, and small-deep. A stream with many pool types will support a wide variety of aquatic species. Rivers with low sinuosity (few bends) and monotonous pool characteristics do not have sufficient quantities and types of habitat to support a diverse aquatic community. General guidelines are any pool dimension (i.e., length, width, oblique) greater than half the cross-section of the stream for separating large from small and 1 m depth separating shallow and deep.

Selected References

Beschta and Platts 1986, USEPA 1983.

Habitat Parameter	Condition Category			
	Optimal	**Suboptimal**	**Marginal**	**Poor**
3b. Pool Variability **(low gradient)**	Even mix of large-shallow, large-deep, small-shallow, small-deep pools present.	Majority of pools large-deep; very few shallow.	Shallow pools much more prevalent than deep pools.	Majority of pools small-shallow or pools absent.
SCORE	20 19 18 17 16	15 14 13 12 11	10 9 8 7 6	5 4 3 2 1 0

3b. Pool Variability—Low Gradient

Optimal Range *(Peggy Morgan, FL DEP)*

Poor Range *(William Taft, MI DNR)*

4 SEDIMENT DEPOSITION

high and low gradient streams

Measures the amount of sediment that has accumulated in pools and the changes that have occurred to the stream bottom as a result of deposition. Deposition occurs from large-scale movement of sediment. Sediment deposition may cause the formation of islands, point bars (areas of increased deposition usually at the beginning of a meander that increase in size as the channel is diverted toward the outer bank) or shoals, or result in the filling of runs and pools. Usually deposition is evident in areas that are obstructed by natural or manmade debris and areas where the stream flow decreases, such as bends. High levels of sediment deposition are symptoms of an unstable and continually changing environment that becomes unsuitable for many organisms.

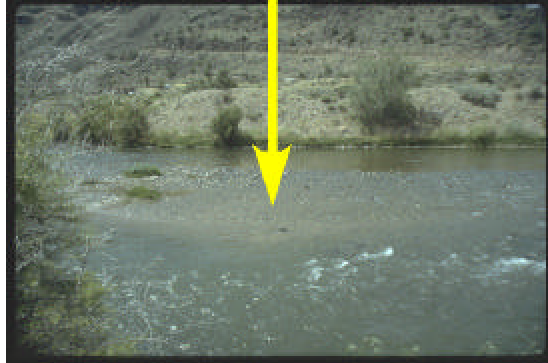
Selected References

MacDonald et al. 1991, Platts et al. 1983, Ball 1982, Armour et al. 1991, Barbour and Stribling 1991, Rosgen 1985.

Habitat Parameter	Condition Category			
	Optimal	**Suboptimal**	**Marginal**	**Poor**
4. Sediment Deposition **(high and low gradient)**	Little or no enlargement of islands or point bars and less than 5% (<20% for low-gradient streams) of the bottom affected by sediment deposition.	Some new increase in bar formation, mostly from gravel, sand or fine sediment; 5-30% (20-50% for low-gradient) of the bottom affected; slight deposition in pools.	Moderate deposition of new gravel, sand or fine sediment on old and new bars; 30-50% (50-80% for low-gradient) of the bottom affected; sediment deposits at obstructions, constrictions, and bends; moderate deposition of pools prevalent.	Heavy deposits of fine material, increased bar development; more than 50% (80% for low-gradient) of the bottom changing frequently; pools almost absent due to substantial sediment deposition.
SCORE	20 19 18 17 16	15 14 13 12 11	10 9 8 7 6	5 4 3 2 1 0

4a. Sediment Deposition—High Gradient

Optimal Range



Poor Range
(arrow pointing to sediment deposition)

4b. Sediment Deposition—Low Gradient

Optimal Range

Poor Range
(arrows pointing to sediment deposition)

5 CHANNEL FLOW STATUS

high and low gradient streams

The degree to which the channel is filled with water. The flow status will change as the channel enlarges (e.g., aggrading stream beds with actively widening channels) or as flow decreases as a result of dams and other obstructions, diversions for irrigation, or drought. When water does not cover much of the streambed, the amount of suitable substrate for aquatic organisms is limited. In high-gradient streams, riffles and cobble substrate are exposed; in low-gradient streams, the decrease in water level exposes logs and snags, thereby reducing the areas of good habitat. Channel flow is especially useful for interpreting biological condition under abnormal or lowered flow conditions. This parameter becomes important when more than one biological index period is used for surveys or the timing of sampling is inconsistent among sites or annual periodicity.

Selected References

Rankin 1991, Rosgen 1985, Hupp and Simon 1986, MacDonald et al. 1991, Ball 1982, Hicks et al. 1991.

Habitat Parameter	Condition Category			
	Optimal	Suboptimal	Marginal	Poor
5. Channel Flow Status **(high and low gradient)**	Water reaches base of both lower banks, and minimal amount of channel substrate is exposed.	Water fills >75% of the available channel; or <25% of channel substrate is exposed.	Water fills 25-75% of the available channel, and/or riffle substrates are mostly exposed.	Very little water in channel and mostly present as standing pools.
SCORE	20 19 18 17 16	15 14 13 12 11	10 9 8 7 6	5 4 3 2 1 0

5a. Channel Flow Status—High Gradient

Optimal Range

Poor Range
(arrow showing that water is not reaching both banks; leaving much of channel uncovered)

5b. Channel Flow Status—Low Gradient

Optimal Range

Poor Range *(James Stahl, IN DEM)*

Parameters to be evaluated broader than sampling reach:

6 CHANNEL ALTERATION

high and low gradient streams

Is a measure of large-scale changes in the shape of the stream channel. Many streams in urban and agricultural areas have been straightened, deepened, or diverted into concrete channels, often for flood control or irrigation purposes. Such streams have far fewer natural habitats for fish, macroinvertebrates, and plants than do naturally meandering streams. Channel alteration is present when artificial embankments, riprap, and other forms of artificial bank stabilization or structures are present; when the stream is very straight for significant distances; when dams and bridges are present; and when other such changes have occurred. Scouring is often associated with channel alteration.

Selected References

Barbour and Stribling 1991, Simon 1989a, b, Simon and Hupp 1987, Hupp and Simon 1986, Hupp 1992, Rosgen 1985, Rankin 1991, MacDonald et al. 1991.

Habitat Parameter	Condition Category			
	Optimal	**Suboptimal**	**Marginal**	**Poor**
6. Channel Alteration **(high and low gradient)**	Channelization or dredging absent or minimal; stream with normal pattern.	Some channelization present, usually in areas of bridge abutments; evidence of past channelization, i.e., dredging, (greater than past 20 yr) may be present, but recent channelization is not present.	Channelization may be extensive; embankments or shoring structures present on both banks; and 40 to 80% of stream reach channelized and disrupted.	Banks shored with gabion or cement; over 80% of the stream reach channelized and disrupted. Instream habitat greatly altered or removed entirely.
SCORE	20 19 18 17 16	15 14 13 12 11	10 9 8 7 6	5 4 3 2 1 0

6a. Channel Alteration—High Gradient

Optimal Range

Poor Range
(arrows emphasizing large-scale channel alterations)

6b. Channel Alteration—Low Gradient

Optimal Range

Poor Range *(John Maxted, DE DNREC)*

7a FREQUENCY OF RIFFLES (OR BENDS)

high gradient streams

Is a way to measure the sequence of riffles and thus the heterogeneity occurring in a stream. Riffles are a source of high-quality habitat and diverse fauna, therefore, an increased frequency of occurrence greatly enhances the diversity of the stream community. For high gradient streams where distinct riffles are uncommon, a run/bend ratio can be used as a measure of meandering or sinuosity (see 7b). A high degree of sinuosity provides for diverse habitat and fauna, and the stream is better able to handle surges when the stream fluctuates as a result of storms. The absorption of this energy by bends protects the stream from excessive erosion and flooding and provides refugia for benthic invertebrates and fish during storm events. To gain an appreciation of this parameter in some streams, a longer segment or reach than that designated for sampling should be incorporated into the evaluation. In some situations, this parameter may be rated from viewing accurate topographical maps. The "sequencing" pattern of the stream morphology is important in rating this parameter. In headwaters, riffles are usually continuous and the presence of cascades or boulders provides a form of sinuosity and enhances the structure of the stream. A stable channel is one that does not exhibit progressive changes in slope, shape, or dimensions, although short-term variations may occur during floods (Gordon et al. 1992).

Selected References

Hupp and Simon 1991, Brussock and Brown 1991, Platts et al. 1983, Rankin 1991, Rosgen 1985, 1994, 1996, Osborne and Hendricks 1983, Hughes and Omernik 1983, Cushman 1985, Bain and Boltz 1989, Gislason 1985, Hawkins et al. 1982, Statzner et al. 1988.

Habitat Parameter	Condition Category			
	Optimal	**Suboptimal**	**Marginal**	**Poor**
7a. Frequency of Riffles (or bends) **(high gradient)**	Occurrence of riffles relatively frequent; ratio of distance between riffles divided by width of the stream <7:1 (generally 5 to 7); variety of habitat is key. In streams where riffles are continuous, placement of boulders or other large, natural obstruction is important.	Occurrence of riffles infrequent; distance between riffles divided by the width of the stream is between 7 to 15.	Occasional riffle or bend; bottom contours provide some habitat; distance between riffles divided by the width of the stream is between 15 to 25.	Generally all flat water or shallow riffles; poor habitat; distance between riffles divided by the width of the stream is a ratio of >25.
SCORE	20 19 18 17 16	15 14 13 12 11	10 9 8 7 6	5 4 3 2 1 0

7a. Frequency of Riffles (or bends)—High Gradient

Optimal Range
(arrows showing frequency of riffles and bends)

Poor Range

7b CHANNEL SINUOSITY

low gradient streams

Evaluates the meandering or sinuosity of the stream. A high degree of sinuosity provides for diverse habitat and fauna, and the stream is better able to handle surges when the stream fluctuates as a result of storms. The absorption of this energy by bends protects the stream from excessive erosion and flooding and provides refugia for benthic invertebrates and fish during storm events. To gain an appreciation of this parameter in low gradient streams, a longer segment or reach than that designated for sampling may be incorporated into the evaluation. In some situations, this parameter may be rated from viewing accurate topographical maps. The "sequencing" pattern of the stream morphology is important in rating this parameter. In "oxbow" streams of coastal areas and deltas, meanders are highly exaggerated and transient. Natural conditions in these streams are shifting channels and bends, and alteration is usually in the form of flow regulation and diversion. A stable channel is one that does not exhibit progressive changes in slope, shape, or dimensions, although short-term variations may occur during floods (Gordon et al. 1992).

Selected References

Hupp and Simon 1991, Brussock and Brown 1991, Platts et al. 1983, Rankin 1991, Rosgen 1985, 1994, 1996, Osborne and Hendricks 1983, Hughes and Omernik 1983, Cushman 1985, Bain and Boltz 1989, Gislason 1985, Hawkins et al. 1982, Statzner et al. 1988.

Habitat Parameter	Condition Category			
	Optimal	**Suboptimal**	**Marginal**	**Poor**
7b. Channel Sinuosity **(low gradient)**	The bends in the stream increase the stream length 3 to 4 times longer than if it was in a straight line. (Note - channel braiding is considered normal in coastal plains and other low-lying areas. This parameter is not easily rated in these areas.)	The bends in the stream increase the stream length 2 to 3 times longer than if it was in a straight line.	The bends in the stream increase the stream length 1 to 2 times longer than if it was in a straight line.	Channel straight; waterway has been channelized for a long distance.
SCORE	20 19 18 17 16	15 14 13 12 11	10 9 8 7 6	5 4 3 2 1 0

7b. Channel Sinuosity—Low Gradient

Optimal Range

Poor Range

8 BANK STABILITY (condition of banks)

high and low gradient streams

Measures whether the stream banks are eroded (or have the potential for erosion). Steep banks are more likely to collapse and suffer from erosion than are gently sloping banks, and are therefore considered to be unstable. Signs of erosion include crumbling, unvegetated banks, exposed tree roots, and exposed soil. Eroded banks indicate a problem of sediment movement and deposition, and suggest a scarcity of cover and organic input to streams. Each bank is evaluated separately and the cumulative score (right and left) is used for this parameter.

Selected References

Ball 1982, MacDonald et al. 1991, Armour et al. 1991, Barbour and Stribling 1991, Hupp and Simon 1986, 1991, Simon 1989a, Hupp 1992, Hicks et al. 1991, Osborne et al. 1991, Rosgen 1994, 1996.

Habitat Parameter	Condition Category			
	Optimal	**Suboptimal**	**Marginal**	**Poor**
8. Bank Stability (score each bank) **Note: determine left or right side by facing downstream** **(high and low gradient)**	Banks stable; evidence of erosion or bank failure absent or minimal; little potential for future problems. <5% of bank affected.	Moderately stable; infrequent, small areas of erosion mostly healed over. 5-30% of bank in reach has areas of erosion.	Moderately unstable; 30-60% of bank in reach has areas of erosion; high erosion potential during floods.	Unstable; many eroded areas; "raw" areas frequent along straight sections and bends; obvious bank sloughing; 60-100% of bank has erosional scars.
SCORE ___ (LB)	Left Bank 10 9	8 7 6	5 4 3	2 1 0
SCORE ___ (RB)	Right Bank 10 9	8 7 6	5 4 3	2 1 0

8a. Bank Stability (condition of banks)—High Gradient

Optimal Range
(arrow pointing to stable streambanks)

Poor Range *(MD Save Our Streams)*
(arrow highlighting unstable streambanks)

8b. Bank Stability (condition of banks)—Low Gradient

Optimal Range *(Peggy Morgan, FL DEP)*

Poor Range
(arrow highlighting unstable streambanks)

9 BANK VEGETATIVE PROTECTION

high and low gradient streams

Measures the amount of vegetative protection afforded to the stream bank and the near-stream portion of the riparian zone. The root systems of plants growing on stream banks help hold soil in place, thereby reducing the amount of erosion that is likely to occur. This parameter supplies information on the ability of the bank to resist erosion as well as some additional information on the uptake of nutrients by the plants, the control of instream scouring, and stream shading. Banks that have full, natural plant growth are better for fish and macroinvertebrates than are banks without vegetative protection or those shored up with concrete or riprap. This parameter is made more effective by defining the native vegetation for the region and stream type (i.e., shrubs, trees, etc.). In some regions, the introduction of exotics has virtually replaced all native vegetation. The value of exotic vegetation to the quality of the habitat structure and contribution to the stream ecosystem must be considered in this parameter. In areas of high grazing pressure from livestock or where residential and urban development activities disrupt the riparian zone, the growth of a natural plant community is impeded and can extend to the bank vegetative protection zone. Each bank is evaluated separately and the cumulative score (right and left) is used for this parameter.

Selected References

Platts et al. 1983, Hupp and Simon 1986, 1991, Simon and Hupp 1987, Ball 1982, Osborne et al. 1991, Rankin 1991, Barbour and Stribling 1991, MacDonald et al. 1991, Armour et al. 1991, Myers and Swanson 1991, Bauer and Burton 1993.

Habitat Parameter	Condition Category			
	Optimal	**Suboptimal**	**Marginal**	**Poor**
9. Vegetative Protection (score each bank) **Note: determine left or right side by facing downstream.** **(high and low gradient)**	More than 90% of the streambank surfaces and immediate riparian zones covered by native vegetation, including trees, understory shrubs, or nonwoody macrophytes; vegetative disruption through grazing or mowing minimal or not evident; almost all plants allowed to grow naturally.	70-90% of the streambank surfaces covered by native vegetation, but one class of plants is not well-represented; disruption evident but not affecting full plant growth potential to any great extent; more than one-half of the potential plant stubble height remaining.	50-70% of the streambank surfaces covered by vegetation; disruption obvious; patches of bare soil or closely cropped vegetation common; less than one-half of the potential plant stubble height remaining.	Less than 50% of the streambank surfaces covered by vegetation; disruption of streambank vegetation is very high; vegetation has been removed to 5 centimeters or less in average stubble height.
SCORE ___ (LB)	Left Bank 10 9	8 7 6	5 4 3	2 1 0
SCORE ___ (RB)	Right Bank 10 9	8 7 6	5 4 3	2 1 0

9a. Bank Vegetative Protection—High Gradient

Optimal Range
(arrow pointing to streambank with high level of vegetative cover)

Poor Range
(arrow pointing to streambank with almost no vegetative cover)

9b. Bank Vegetative Protection—Low Gradient

Optimal Range *(Peggy Morgan, FL DEP)*

Poor Range *(MD Save Our Streams)*
(arrow pointing to channelized streambank with no vegetative cover)

10 RIPARIAN VEGETATIVE ZONE WIDTH

high and low gradient streams

Measures the width of natural vegetation from the edge of the stream bank out through the riparian zone. The vegetative zone serves as a buffer to pollutants entering a stream from runoff, controls erosion, and provides habitat and nutrient input into the stream. A relatively undisturbed riparian zone supports a robust stream system; narrow riparian zones occur when roads, parking lots, fields, lawns, bare soil, rocks, or buildings are near the stream bank. Residential developments, urban centers, golf courses, and rangeland are the common causes of anthropogenic degradation of the riparian zone. Conversely, the presence of "old field" (i.e., a previously developed field not currently in use), paths, and walkways in an otherwise undisturbed riparian zone may be judged to be inconsequential to altering the riparian zone and may be given relatively high scores. For variable size streams, the specified width of a desirable riparian zone may also be variable and may be best determined by some multiple of stream width (e.g., 4 x wetted stream width). Each bank is evaluated separately and the cumulative score (right and left) is used for this parameter.

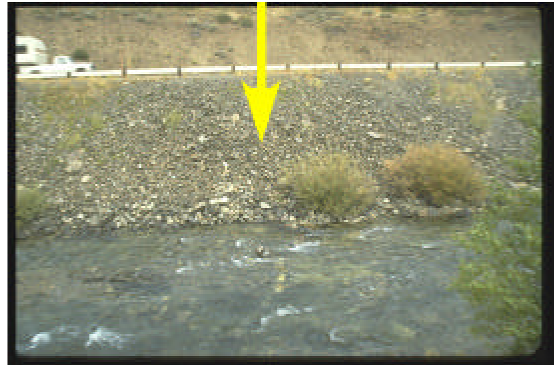
Selected References

Barton et al. 1985, Naiman et al. 1993, Hupp 1992, Gregory et al. 1991, Platts et al. 1983, Rankin 1991, Barbour and Stribling 1991, Bauer and Burton 1993.

Habitat Parameter	Condition Category			
	Optimal	**Suboptimal**	**Marginal**	**Poor**
10. Riparian Vegetative Zone Width (score each bank riparian zone) **(high and low gradient)**	Width of riparian zone >18 meters; human activities (i.e., parking lots, roadbeds, clear-cuts, lawns, or crops) have not impacted zone.	Width of riparian zone 12-18 meters; human activities have impacted zone only minimally.	Width of riparian zone 6-12 meters; human activities have impacted zone a great deal.	Width of riparian zone <6 meters: little or no riparian vegetation due to human activities.
SCORE ___ (LB)	Left Bank 10 9	8 7 6	5 4 3	2 1 0
SCORE ___ (RB)	Right Bank 10 9	8 7 6	5 4 3	2 1 0

10a. Riparian Vegetative Zone Width—High Gradient

Optimal Range
(arrow pointing out an undisturbed riparian zone)



Poor Range
(arrow pointing out lack of riparian zone)

10b. Riparian Vegetative Zone Width—Low Gradient

Optimal Range
(arrow emphasizing an undisturbed riparian zone)

Poor Range *(MD Save Our Streams)*
(arrow emphasizing lack of riparian zone)

5.3 ADDITIONS OF QUANTITATIVE MEASURES TO THE HABITAT ASSESSMENT

Kaufmann (1993) identified 7 general physical habitat attributes important in influencing stream ecology. These include:

- channel dimensions
- channel gradient
- channel substrate size and type
- habitat complexity and cover
- riparian vegetation cover and structure
- anthropogenic alterations
- channel-riparian interaction.

All of these attributes vary naturally, as do biological characteristics; thus expectations differ even in the absence of anthropogenic disturbances. Within a given physiographic-climatic region, stream drainage area and overall stream gradient are likely to be strong natural determinants of many aspects of stream habitat, because of their influence on discharge, flood stage, and stream power (the product of discharge times gradient). In addition, all of these attributes may be directly or indirectly altered by anthropogenic activities.

In Section 5.2, an approach is described whereby habitat quality is interpreted directly in the field by biologists while sampling the stream reach. This Level 1 approach is observational and requires only one person (although a team approach is recommended) and takes about 15 to 20 minutes per stream reach. This approach more quickly yields a habitat quality assessment. However, it depends upon the knowledge and experience of the field biologist to make the proper interpretation of observed of both the natural expectations (potentials) and the biological consequences (quality) that can be attributed to the observed physical attributes. Hannaford et al. (1997) found that training in habitat assessment was necessary to reduce the subjectivity in a visual-based approach. The authors also stated that training on different types of streams may be necessary to adequately prepare investigators.

The second conceptual approach described here confines observations to habitat characteristics themselves (whether they are quantitative or qualitative), then later ascribing quality scoring to these measurements as part of the data analysis process. Typically, this second type of habitat assessment approach employs more quantitative data collection, as exemplified by field methods described by Kaufmann and Robison (1997) for EMAP, Simonson et al. (1994), Meador et al. (1993) for NAWQA, and others cited by Gurtz and Muir (1994). These field approaches typically define a reach length proportional to stream width and employ transect measurements that are systematically spaced (Simonson et al. 1994, Kaufmann and Robison 1997) or spaced by judgement to be representative (Meador et al. 1993). They usually include measurement of substrate, channel and bank dimensions, riparian canopy cover, discharge, gradient, sinuosity, in-channel cover features, and counts of large woody debris and riparian human disturbances. They may employ systematic visual estimates of substrate embeddedness, fish cover features, habitat

types, and riparian vegetation structure. The time commitment in the field to these more quantitative habitat assessment methods is usually 1.5 to 3 hours with a crew of two people. Because of the greater amount of data collected, they also require more time for data summarization, analysis, and interpretation. On the other hand, the more quantitative methods and less ambiguous field parameters result in considerably greater precision. The USEPA applied both quantitative and visual-based (RBPs) methods in a stream survey undertaken over 4 years in the mid-Atlantic region of the Appalachian Mountains. An earlier version of the RBP techniques were applied on 301 streams with repeat visits to 29 streams; signal-to-noise ratios varied from 0.1 to 3.0 for the twelve RBP metrics and averaged (1.1 for the RBP total habitat quality score). The quantitative methods produced a higher level of precision; signal-to-noise ratios were typically between 10 and 50, and sometimes in excess of 100 for quantitative measurements of channel morphology, substrate, and canopy densiometer measurements made on a random subset of 186 streams with 27 repeat visits in the same survey. Similarly, semi-quantitative estimates of fish cover and riparian human disturbance estimates obtained from multiple, systematic visual observations of otherwise measurable features had signal:noise ratios from 5 to 50. Many riparian vegetation cover and structure metrics were moderately precise (signal:noise ranging from 2 to 30). Commonly used flow dependent measures (e.g., riffle/pool and width/depth ratios), and some visual riparian cover estimates were less precise, with signal:noise ratios more in the range of those observed for metrics of the EPA's RBP habitat score (<2).

The USEPA's EMAP habitat assessment field methods are presented as an option for a second level (II) of habitat assessment. These methods have been applied in numerous streams throughout the Mid-Atlantic region, the Midwest, Colorado, California, and the Pacific Northwest. Table 5-1 is a summary of these field methods; more detail is presented in the field manual by Kaufmann and Robison (1997).

Table 5-1. Components of EMAP physical habitat protocol.

Component	Description
1. Thalweg Profile	Measure maximum depth, classify habitat, determine presence of soft/small sediment at 10-15 equally spaced intervals between each of 11 channel cross-sections (100-150 along entire reach). Measure wetted width at 11 channel cross-sections and mid-way between cross-sections (21 measurements).
2. Woody Debris	Between each of the channel cross sections, tally large woody debris numbers within and above the bankfull channel according to size classes.
3. Channel and Riparian Cross-Sections	At 11 cross-section stations placed at equal intervals along reach length: • **Measure**: channel cross section dimensions, bank height, undercut, angle (with rod and clinometer); gradient (clinometer), sinuosity (compass backsite), riparian canopy cover (densiometer). • **Visually Estimate***: substrate size class and embeddedness; areal cover class and type (e.g., woody) of riparian vegetation in Canopy, Mid-Layer and Ground Cover; areal cover class of fish concealment features, aquatic macrophytes and filamentous algae. • **Observe & Record***: human disturbances and their proximity to the channel.
4. Discharge	In medium and large streams (defines later) measure water depth and velocity @ 0.6 depth (with electromagnetic or impeller-type flow meter) at 15 to 20 equally spaced intervals across one carefully chosen channel cross-section. In very small streams, measure discharge with a portable weir or time the filling of a bucket.

* Substrate size class and embeddedness are estimated, and depth is measured for 55 particles taken at 5 equally-spaced points on each of 11 cross-sections. The cross-section is defined by laying the surveyor's rod or tape to span the wetted channel. Woody

debris is tallied over the distance between each cross-section and the next cross-section upstream. Riparian vegetation and human disturbances are observed 5 m upstream and 5 m downstream from the cross section station. They extend shoreward 10 m from left and right banks. Fish cover types, aquatic macrophytes, and algae are observed within channel 5 m upstream and 5 m downstream from the cross section stations. These boundaries for visual observations are estimated by eye.

Table 5-2 lists the physical habitat metrics that can be derived from applying these field methods. Once these habitat metrics are calculated from the available physical habitat data, an assessment would be obtained from comparing these metric values to those of known reference sites. A strong deviation from the reference expectations would indicate a habitat alteration of the particular parameter. The close connectivity of the various attributes would most likely result in an impact on multiple metrics if habitat alteration was occurring. The actual process for interpreting a habitat assessment using this approach is still under development.

Table 5-2. Example of habitat metrics that can be calculated from the EMAP physical habitat data.

Channel mean width and depth
Channel volume and Residual Pool volume
Mean channel slope and sinuosity
Channel incision, bankfull dimensions, and bank characteristics
Substrate mean diameter, % fines, % embeddedness
Substrate stability
Fish concealment features (areal cover of various types, e.g., undercut banks, brush)
Large woody debris (volume and number of pieces per 100 m)
Channel habitat types (e.g., % of reach composed of pools, riffles, etc.)
Canopy cover
Riparian vegetation structure and complexity
Riparian disturbance measure (proximity-weighted tally of human disturbances)

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<u>Section 2: Stream Site Information</u>

Contents

Site Selection Criteria......2

Stream Site Naming Convention......3

Determining Your Site's Waypoints......4

Site Selection Criteria

For the most part, your research focus will dictate your stream site selection. In general, your monitoring sites will likely follow these guidelines:

- Sites selected should preferably be 2nd or 3rd order streams.
- Avoid meanders, and look to areas with riffles.
- Make sure that you have easy access to the stream sites. They should not be too far from a road, and should not require crossing private property.
- If it works for your research, we encourage you to monitor sites that have been monitored in previous years by the Streams Project though this is by no means required. Speak with Streams Project staff about opportunities here.

Stream Site Naming Convention

*It is very important that any site you will be sampling for the Streams Project has been given a stream site name with the naming convention outlined here. While the process for deriving a stream site name is explained here, you must confirm your stream site name with Streams Project staff (*streams@uvm.edu).

1. When you first go out into the field to choose your monitoring site, or on your first visit, take a GPS point following the guidelines outlined in the document on how to collect waypoints (pages 2-4 and 2-5 in this manual). The guide to collecting waypoints requires that you collect both coordinates in decimal degrees and a measurement of elevation in feet.

2. Your site code will follow the format outlined in the box below. You will need to email Streams Project staff *(*streams@uvm.edu)to get the first digits of your stream site code, but the elevation you will have from your first visit in the field:

3. **IMPORTANT:** Make sure to use your stream site codes on ***ALL*** your field data records, and on all samples you send to either the water quality or macroinvertebrate labs.

GENERAL FORMAT FOR STREAM SITE CODE:

HUC8WatershedAbbreviation_StreamNameAbbreviation_Elevation*

Red = elevation and feet read from GPS unit in the field

Blue = the first digits of code emailed to you by Streams Project staff

* The underscores ("_") are part of the site code

EXAMPLE:

The stream site code for Munroe Brook in the Lake Champlain Direct HUC8 watershed, with a GPS field elevation reading of 500 feet:

SITE CODE = LCD_MuBr_500

Determining Your Site's Waypoints

Field Method: Taking and Downloading Waypoints from GPS Units for your Sites

*Some of you may have used GPS units before, and to some of you it may be entirely new. Either way, **PLEASE** read the procedure outline here for collecting way points in the field. To prevent unnecessary conversions after collection, the Streams Project has outlined here a standard setup for your GPS unit as well as details how to use your GPS unit to collect waypoints if this is a new concept.*

Before going out into the field:

1. Turn GPS unit on– power button on right side of GPS units.

2. Click the button on the top right side of the GPS unit until a menu page appears with an icon and the word **setup** as an option. Scroll* to **setup** and select.

3. Under the setup menu scroll to **units** and select.

4. In the units menu, make sure that the position format is set to **hddd.ddddd°** (decimal degrees) and the map datum is set to **WGS 84.** The elevation should be set to feet.

In the field:

5. Turn GPS unit on

6. Wait for the GPS unit to register the satellites (For the green EPSCoR GPS unit you might have to hit the menu button or front button once or twice).

7. Once the satellites have registered and you are ready to mark your site, hold the button down on the front of the GPS unit until a screen pops up describing your waypoint (point referencing your site location).

8. Record the elevation in feet from this screen for your stream site code.

9. Scroll to where the GPS unit has named this waypoint – usually a number. You can change this number, so change it to the stream site code if you've already determined what that is. ***NOTE:** The GPS units do not have an underscore (_) so instead use the dash sign (-).*

10. ***If you have the gray GPS unit***, to the left of the screen will be a button called **Avg**. Select this button. At the bottom of the screen that comes up you will see that that the **Measurement Count** is increasing steadily – the higher the point count, the more measurements have been taken (and then averaged) for your location to increase precision. You will also note that the **Estimated Accuracy** tab fluctuates as well. The accuracy tells you within how many feet of your real location the satellites have put this point.

11. Hit save, and then ok. Your point is now recorded in the GPS Unit.

12. Downloading the points is slightly more complicated – please set up a time to meet with Streams Project staff to download your points or get more information on how to do this on your own (streams@uvm.edu)

* Use the front button on the GPS unit – to scroll you push it side to side, to select press the button in. If your GPS unit does not have this front button there are likely arrow buttons on the side to scroll up and down, and an enter button.

Section 3: Water Quality

Contents

Water Quality Assessment General Info 2

Field Equipment Checklist 3

Water Quality Monitoring Background Information 4

In-Stream Measurements 7

Stream Flow & Discharge 7

Temperature 9

pH 11

Field Method: Flow & Discharge 13

Field Method: Temperature 15

Field Method: pH 16

Data Form For Calculating Flow 18

Water Quality Assessment Data Sheet 19

Water Samples 20

Bacteria 20

Phosphorus 23

Total Suspended Solids (TSS) 27

Field Method: Water Samples for Bacteria, TSS & Phosphorus 29

Water Quality Field Labels 33

Water Quality Laboratory Methods 34

Lab Method: Determination of Bacteria Using IDEXX Colilert System 34

Lab Method: Determination of Total Suspended Solids (TSS) 35

Lab Method: Determination of Total Phosphorus 37

Water Quality Assessment General Info

There are several components to water quality assessment:

- Discharge or flow
- Temperature
- pH
- Water samples for: bacteria, TSS, and/or phosphorus

Undergraduates: Take discharge, temperature, and pH measurements each time you visit a site, regardless of what other types of samples you are collecting. Use the "WATER QUALITY ASSESSMENT DATA SHEET".

High School Teams: Take all the components listed above each time you visit a stream site. You should visit each stream site every two weeks. Use the "WATER QUALITY ASSESSMENT DATA SHEET".

All Participants: The data sheet and field labels that you need to take with you in the field are embedded throughout this section and there are extra copied in the "Extra Data Sheets" section of your manual. The data that you collect and record in the field on your "Water Quality Assessment Data Sheet" should be entered online by you as soon as possible after your site visit.

The lab methods and protocols are included at the end of this section. Most of you will not reference these protocols. They are included here so that all participants may understand how the water samples are processed when they are sent to the Water Quality Lab at UVM.

Field Equipment Checklist

General Equipment

- Waders
- Spray bottles with disinfectant solution and water
- Gloves
- Pencils and permanent marker
- Data entry form (one for each site)
- Clipboard
- Insect repellent/sunscreen
- Sunglasses
- Camera
- First Aid kit
- Cell phone

Physical parameters

- Meter tape
- Meter stick
- Water temperature/pH meter
- Stopwatch
- Tennis ball

Water samples (undergraduates may not need all of these samples)

- 3 bottles for TP: labeled with site code, date, and total phosphorus
- 3 bottles for *E.coli*: labeled with site code, date and *E. coli*
- 3 bottles for TSS: labeled with site code, date and TSS
- Cooler with ice
- Extra bottles and labels

Water Quality Monitoring Background Information

What is water quality monitoring?

For our purposes, we define water quality monitoring as the sampling and analysis of water constituents and conditions. These may include:

- Introduced pollutants, such as pesticides, metals, and oil
- Constituents found naturally in water that can nevertheless be affected by human sources, such as dissolved oxygen, bacteria, and nutrients

The magnitude of their effects can be influenced by properties such as pH and temperature. For example, temperature influences the quantity of dissolved oxygen that water is able to contain, and pH affects the toxicity of ammonia.

What part of water quality does the Streams Project monitor?

The Streams Project collects water quality data on temperature, pH, phosphorus (an important nutrient for primary production), total suspended solids, and bacteria. The Streams Project also collects information on macroinvertebrate communities, which are longer-term indicators of water quality, whereas water samples (i.e. temperature, pH, phosphorus, TSS, bacteria) are a snapshot of water quality at a specific sampling moment.

The data that the Streams Project collects are used for the purposes detailed below as well as the individual research projects of high school teams and undergraduate interns.

Who monitors water quality?

Local and national water quality professionals, volunteers, and researchers, have been monitoring water quality conditions for many years. In fact, until the past decade or so (when biological monitoring protocols were developed and began to take hold), water quality monitoring was generally considered the primary way of identifying water pollution problems. Today, professional water quality specialists and volunteer program coordinators alike are moving toward approaches that combine chemical, physical, and biological monitoring methods to achieve the best picture of water quality conditions.

Continued….

Government agencies have searchable water quality databases. Below are a few of the larger publicly-available databases:

- USEPA Storage and retrieval data base (STORET):
  - www.epa.gov/storet/
- US Geological Survey: Fixed monitoring stations for hydrology and water quality monitoring
  - waterdata.usgs.gov/nwis/qw
- National Oceanic and Atmospheric Administration (NOAA):
  - Sea Grant Program (Lake Champlain & Great Lakes)
  - National Status and Trends Program
  - www.research.noaa.gov/oceans/t_hydrology.html
- State agencies
  - VT Department of Environmental Conservation: www.anr.state.vt.us/dec/dec.htm
    → For data go to www.vtwaterquality.org/wqd_mgtplan/waterq_data.htm
  - VT Department of Fish & Wildlife: www.vtfishandwildlife.com
- University research reports and journal articles

Why monitor water quality?

Water quality monitoring can be used for many purposes:

- *To identify whether waters are meeting designated uses.* All states have established specific criteria (limits on pollutants) identifying what concentrations of chemical pollutants are allowable in their waters. When chemical pollutants exceed maximum or minimum allowable concentrations, waters may no longer be able to support the beneficial uses such as fishing, swimming, and drinking for which they have been designated. Designated uses and the specific criteria that protect them (along with antidegradation statements say waters should not be allowed to deteriorate below existing or anticipated uses) together form water quality standards. State water quality professionals assess water quality by comparing the concentrations of chemical pollutants found in streams to the criteria in the state's standards, and so judge whether streams are meeting their designated uses.

  Water quality monitoring, however, might be inadequate for determining whether aquatic life uses are being met in a stream. While some constituents (such as dissolved oxygen and temperature) are important to maintaining healthy fish and aquatic insect populations, other factors, such as the physical structure of the stream and the condition of the habitat, play an equal or greater role. Biological monitoring methods are generally better suited to determining whether aquatic life is supported.

- *To identify specific pollutants and sources of pollution.* Water quality monitoring helps link sources of pollution to a stream quality problem because it identifies specific problem pollutants. Since certain activities tend to generate certain

pollutants (e.g., bacteria and nutrients are more likely to come from an animal feedlot than an automotive repair shop), a tentative link might be made that would warrant further investigation or monitoring.

- *To determine trends.* Chemical constituents that are properly monitored (i.e., consistent time of day and on a regular basis, using consistent methods) can be analyzed for trends over time.

- *To screen for impairment.* Finding excessive levels of one or more chemical constituents can serve as an early warning "screen" of potential pollution problems.

Table 1 describes sources of water quality degradation and the types of pollutants associated with each source.

Source	Common Associated Chemical Pollutants
Cropland	Turbidity, phosphorus, nitrates, temperature, total solids
Forestry harvest	Turbidity, temperature, total solids
Grazing land	Fecal bacteria, turbidity, phosphorus, nitrates, temperature
Industrial discharge	Temperature, conductivity, total solids, toxics, pH
Mining	pH, alkalinity, total dissolved solids
Septic systems	Fecal bacteria (i.e., Escherichia coli, enterococcis), nitrates, phosphorus, dissolved oxygen/biochemical oxygen demand, conductivity, temperature
Sewage treatment plants	Dissolved oxygen and biochemical oxygen demand, turbidity, conductivity, phosphorus, nitrates, fecal bacteria, temperature, total solids, pH
Construction	Turbidity, temperature, dissolved oxygen and biochemical oxygen demand, total solids, and toxics
Urban runoff	Turbidity, phosphorus, nitrates, temperature, conductivity, dissolved oxygen and biochemical oxygen demand

Table 1

Sources and associated pollutants
A volunteer water quality monitoring program should be geared to the types of watershed land uses most often encountered.

In-Stream Measurements

Stream Flow & Discharge

What is stream flow and why is it important?

Stream flow, or discharge, is the **volume** of water that moves over a designated point over a fixed period of time. It is often expressed as cubic feet per second (ft^3/sec).

The flow of a stream is directly related to the amount of water moving off the watershed into the stream channel. It is affected by weather, increasing during rainstorms and decreasing during dry periods. It also changes during different seasons of the year, decreasing during the summer months when evaporation rates are high and shoreline vegetation is actively growing and removing water from the ground. August and September are usually the months of lowest flow for most streams and rivers in most of the country.

Water withdrawals for irrigation purposes can seriously deplete water flow, as can industrial water withdrawals. Dams used for electric power generation, particularly facilities designed to produce power during periods of peak need, often block the flow of a stream and later release it in a surge.

Flow is a function of water volume and velocity. It is important because of its impact on water quality and on the living organisms and habitats in the stream. Large, swiftly flowing rivers can receive pollution discharges and be little affected, whereas small streams have less capacity to dilute and degrade wastes.

Stream velocity, which increases as the volume of the water in the stream increases, determines the kinds of organisms that can live in the stream (some need fast-flowing areas; others need quiet pools). It also affects the amount of silt and sediment carried by the stream. Sediment introduced to quiet, slow-flowing streams will settle quickly to the stream bottom. Fast moving streams will keep sediment suspended longer in the water column. Lastly, fast-moving streams generally have higher levels of dissolved oxygen than slow streams because they are better aerated.

This section describes one method for estimating flow in a specific area or reach of a stream. It is adapted from techniques used by several monitoring programs and uses a float (an object such as an orange, ping-pong ball, pine cone, etc.) to measure stream velocity. Calculating flow involves solving an equation that examines the relationship among several variables including stream cross-sectional area, stream length, and water velocity. One way to measure flow is to solve the following equation:

$$\text{Flow} = ALC / T$$

Where:

A = Average cross-sectional area of the stream (stream width multiplied by average water depth).

L = Length of the stream reach measured (usually 20 ft.)

C = A coefficient or correction factor (0.8 for rocky-bottom streams or 0.9 for muddy-bottom streams). This allows you to correct for the fact that water at the surface travels faster than near the stream bottom due to resistance from gravel, cobble, etc. Multiplying the surface velocity by a correction coefficient decreases the value and gives a better measure of the stream's overall velocity.

T = Time, in seconds, for the float to travel the length of L

References

Adopt-A-Stream Foundation. *Field Guide: Watershed Inventory and Stream Monitoring Methods,* by Tom Murdoch and Martha Cheo. 1996. Everett, WA.

Mitchell, M.K., and W. Stapp. *Field Manual for Water Quality Monitoring.* 5th Edition. Thompson Shore Printers.

Missouri Stream Teams. *Volunteer Water Quality Monitoring.* Missouri Department of Natural Resources, P.O. Box 176, Jefferson City, MO 65102.

Temperature

Why is temperature important?

The rates of biological and chemical processes depend on temperature. Aquatic organisms from microbes to fish are dependent on certain temperature ranges for their optimal health. Optimal temperatures for fish depend on the species: some survive best in colder water, whereas others prefer warmer water. Benthic macroinvertebrates are also sensitive to temperature and will move in the stream to find their optimal temperature. If temperatures are outside this optimal range for a prolonged period of time, organisms are stressed and can die. Temperature is measured in de-grees Fahrenheit (F) or degrees Celsius (C).

For fish, there are two kinds of limiting temperatures the maximum temperature for short exposures and a weekly average temperature that varies according to the time of year and the life cycle stage of the fish species. Reproductive stages (spawning and embryo development) are the most sensitive stages. Table 5.5 provides temperature criteria for some species.

Temperature affects the oxygen content of the water (oxygen levels become lower as temperature increases); the rate of photosynthesis by aquatic plants; the metabolic rates of aquatic organisms; and the sensitivity of organisms to toxic wastes, parasites, and diseases.

Causes of temperature change include weather, removal of shading streambank vegetation, impoundments (a body of water confined by a barrier, such as a dam), dis-charge of cooling water, urban storm water, and groundwater inflows to the stream.

Sampling and Equipment Considerations

Temperature in a stream will vary with width and depth. It can be significantly different in the shaded portion of the water on a sunny day. In a small stream, the temperature will be relatively constant as long as the stream is uniformly in sun or shade. In a large stream, temperature can vary considerably with width and depth regardless of shade. If it is safe to do so, temperature measurements should be collected at varying depths and across the surface of the stream to obtain vertical and horizontal temperature profiles. This can be done at each site at least once to determine the necessity of collecting a profile during each sampling visit. Temperature should be measured at the same place every time.

Temperature is measured in the stream with a thermometer or a meter. Alcohol-filled thermometers are preferred over mercury-filled because they are less hazardous if broken. Armored thermometers for field use can withstand more abuse than unprotected glass thermometers and are worth the additional expense. Meters for other tests, such as pH (acidity) or dissolved oxygen, also measure temperature and can be used instead of a thermometer.

Table 5.5

Maximum average temperatures for growth and short-term maximum temperatures for selected fish (°C and °F)

(Brungs and Jones 1977)

Species	Max. weekly average temp. for growth (juveniles)	Max. temp. for survival of short exposure (juveniles)	Max. weekly average temp. for spawning [a]	Max. temp. for embryo spawning [b]
Atlantic salmon	20 °C (68 °F)	23 °C (73 °F)	5 °C (41 °F)	11 °C (52 °F)
Bluegill	32 °C (90 °F)	35 °C (95 °F)	25 °C (77 °F)	34 °C (93 °F)
Brook trout	19 °C (66 °F)	24 °C (75 °F)	9 °C (48 °F)	13 °C (55 °F)
Common carp	---	---	21 °C (70 °F)	33 °C (91 °F)
Channel catfish	32 °C (90 °F)	35 °C (95 °F)	27 °C (81 °F)	29 °C (84 °F)
Largemouth bass	32 °C (90 °F)	34 °C (93 °F)	21 °C (70 °F)	27 °C (81 °F)
Rainbow trout	19 °C (66 °F)	24 °C (75 °F)	9 °C (48 °F)	13 °C (55 °F)
Smallmouth bass	29 °C (84 °F)	---	17 °C (63 °F)	23 °C (73 °F)
Sockeye salmon	18 °C (64 °F)	22 °C (72 °F)	10 °C (50 °F)	13 °C (55 °F)

a - Optimum or mean of the range of spawning temperatures reported for the species
b - Upper temperature for successful incubation and hatching reported for the species
c - Upper temperature for spawning

pH

What Is pH and why is it important?

pH is a term used to indicate the alkalinity or acidity of a substance as ranked on a scale from 1.0 to 14.0. Acidity increases as the pH gets lower. Fig. 5.9 presents the pH of some common liquids.

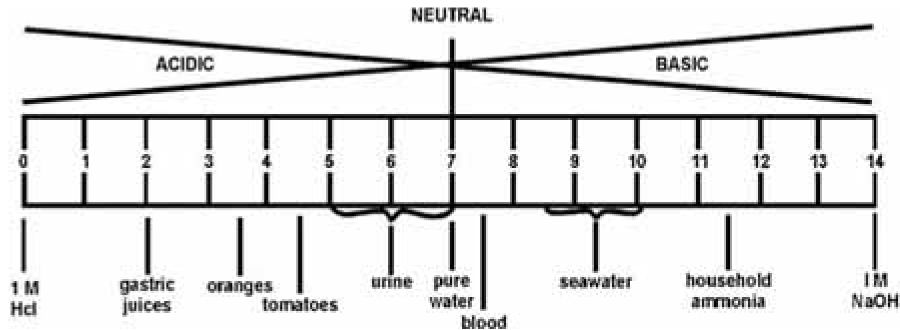


Figure 5.9

pH of selected liquids

pH affects many chemical and biological processes in the water. For example, different organisms flourish within different ranges of pH. The largest variety of aquatic animals prefer a range of 6.5-8.0. pH outside this range reduces the diversity in the stream because it stresses the physiological systems of most organisms and can reduce reproduction. Low pH can also allow toxic elements and compounds to become mobile and "available" for uptake by aquatic plants and animals. This can produce conditions that are toxic to aquatic life, particularly to sensitive species like trout. Changes in acidity can be caused by atmospheric deposition (acid rain), surrounding rock, and certain wastewater discharges.

The pH scale measures the logarithmic concentration of hydrogen (H+) and hydroxide (OH-) ions, which make up water (H+ + OH- = H2O). When both types of ions are in equal concentration, the pH is 7.0 or neutral. Below 7.0, the water is acidic (there are more hydrogen ions than hydroxide ions). When the pH is above 7.0, the water is alkaline, or basic (there are more hydroxide ions than hydrogen ions). Since the scale is logarithmic, a drop in the pH by 1.0 unit is equivalent to a 10-fold increase in acidity. So, a water sample with a pH of 5.0 is 10 times as acidic as one with a pH of 6.0, and pH 4.0 is 100 times as acidic as pH 6.0.

Analytical and equipment considerations

pH can be analyzed in the field or in the lab. If it is analyzed in the lab, you must measure the pH within 2 hours of the sample collection. This is because the pH will change due to the carbon dioxide from the air dissolving in the water, which will bring the pH toward 7.

pH Meters

A pH meter measures the electric potential (millivolts) across an electrode when immersed in water. This electric potential is a function of the hydrogen ion activity in the sample. Therefore, pH meters can display results in either millivolts (mV) or pH units.

A pH meter consists of a *potentiometer,* which measures electric current; a glass electrode, which senses the electric potential where it meets the water sample; a reference electrode, which provides a constant electric potential; and a temperature compensating device, which adjusts the readings according to the temperature of the sample (since pH varies with temperature). The reference and glass electrodes are frequently combined into a single probe called a combination electrode.

The most important part of the pH meter is the electrode. Follow the manufacturer's instructions for proper maintenance. Infrequently used or improperly maintained electrodes are subject to corrosion, which makes them highly inaccurate.

Field Method: Flow & Discharge

I. Introduction

Discharge, or stream flow, is the volume of water that moves over a designated point over a fixed period of time. The rate of discharge is expressed in cubic meters per second (m^3/sec) and is calculated using measurements of stream width, depth, and velocity.

A stream's discharge is directly affected by factors such as precipitation, riparian vegetation, and surrounding land use. The volume and velocity of the water directly impacts water quality and a stream's ability to support macroinvertebrate life.

The average cross-sectional area of a stream reach is determined by measuring the total stream width and stream depth along two transects. Stream velocity is determined by measuring the time it takes a tennis ball to travel the length of a stream reach. Stream flow is calculated from these variables according to the equation Flow = (A x L x C) / T.

II. Equipment and Materials

a. Meter tape
b. Meter stick
c. Stopwatch
d. Tennis ball
e. Waders
f. Data entry form
g. Clipboard and pencil
h.

III. Average Cross-Sectional Area

a. Transect #1
   i. Using your measuring tape, designate the upstream end of your stream reach by stretching the measuring tape across the stream perpendicular to the stream banks.
   ii. Measure the width of the stream from wetted edge to wetted edge. Record as the Total Width in meters under Transect #1 (Upstream) on the data entry form.
   iii. Divide the Total Width into four equal intervals. Record as the Interval Width from Point A to B, Point B to C….D to E.
      1. Example: Total Width=12 meters→Intervals= 3 meters
   iv. Measure the water depth at each interval point (see Figure 1) and record as the depth in meters. Interval E is the shoreline, so its depth may be 0 meters.
   v. Follow the calculations on the data entry form to determine the Cross-Sectional Area of Transect #1.
b. Designate Reach Length
   i. Using your measuring tape, measure 6 meters (~20 feet) downstream from Transect #1. Record as the Length of Stream Reach (L) in meters.
   ii. The downstream end will be Transect #2.

c. Transect #2
   i. Follow the steps provided above for Transect #1 and record all values under Transect #2 (Downstream) on the data entry form.
d. Calculate the Cross-Sectional Area of Transect #2.
e. Following the directions on the data entry form to calculate the Average Cross-Sectional Area (A) of the stream reach

IV. Travel Time

a. Position one researcher above Transect #1 with the tennis ball. A second researcher should be positioned below Transect #2 ready to catch the tennis ball as it travels downstream.
b. The upstream researcher should drop the tennis ball slightly upstream of Transect #1. Position the ball so that it will travel along the fastest current.
c. Using a stopwatch, begin timing when the tennis ball passes Transect #1 and stop timing when the ball completely passes Transect #2. The downstream researcher should catch the tennis ball.
d. Record the time as the Travel Time (T) in seconds.
e. Repeat steps a-d for a total of at least three trials.
f. Calculate the Average Travel Time (T) in seconds.

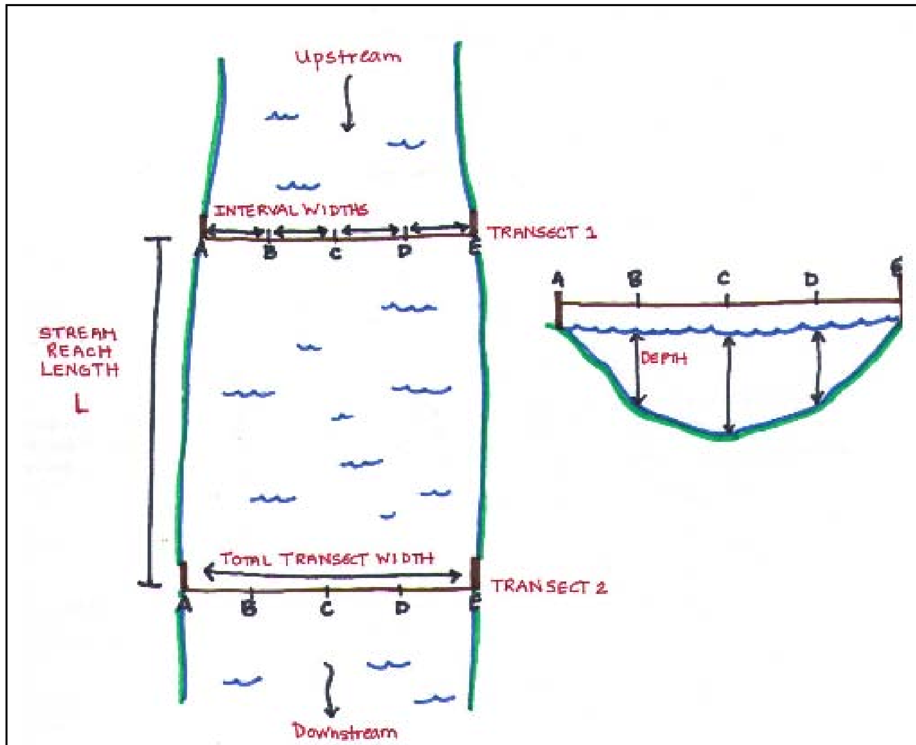
V. Specify the Coefficient or the Correction Factor (C)

a. Enter 0.8 for rocky-bottom streams or 0.9 for sandy-bottom streams

VI. Calculate Discharge

a. Flow = (A x L x C)/T

Figure 1: Discharge Diagram



Field Method: Temperature

The procedures for measuring temperature consist of the following tasks.

TASK 1 Prepare before leaving for the sampling site

Note time, safety considerations, checking supplies, and checking weather and directions. In addition to the standard sampling equipment and apparel, when measuring temperature you will need:

- A thermometer or meter
- A data sheet for temperature to record results

Be sure to let someone know where you are going and when you expect to return

TASK 2 Measure the temperature

In general, sample away from the streambank in the main current. The outside curve of the stream is often a good place to sample since the main current tends to hug this bank. In shallow stretches, wade into the center current carefully to measure temperature. If wading to the center current is not possible, reach out from the shore as far as **safely** possible.

Measure temperature as follows:

1. Place the thermometer or meter probe in the water as least 4 inches below the surface or halfway to the bottom if in a shallow stream.
2. If using a thermometer, allow enough time for it to reach a stable temperature (at least 1 minute). If using a meter, allow the temperature reading to stabilize at a constant temperature reading.
3. If possible, try to read the temperature with the thermometer bulb beneath the water surface. If it is not possible, quickly remove the thermometer and read the temperature.
4. Record the temperature on the field data sheet.

TASK 3 Return the field data sheets to the lab/dropoff point.

References

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Field Method: pH

The field procedures for collecting and analyzing samples for pH consist of the following tasks.

TASK 1 Prepare the sample containers

Sample containers (and all glassware used in this procedure) must be cleaned and rinsed before the first run and after each sampling run by following the procedure described under Method A on page 128. Remember to wear latex gloves.

TASK 2 Prepare before leaving for the sampling site

Refer to Section 2.3 - Saftey Considerations for details on confirming sampling date and time, picking up and checking supplies, and checking weather and directions. In addition to the standard sampling equipment and apparel, when sampling for pH, include the following equipment:

- pH meter with combination temperature and reference electrode, or pH "pocket pal" or color comparator
- Wash bottle with deionized water to rinse pH meter electrode (if appropriate)
- Data sheet for pH to record results

Before you leave for the sampling site, be sure to calibrate the pH meter or "pocket pal." The pH meter and "pocket pal" should be calibrated prior to sample analysis and after every 25 samples according to the instructions that come with them.

If you are using a "pocket pal," use the buffer recommended by the manufacturer. If you are using a laboratory grade meter, use two pH standard buffer solutions: 4.01 and 7.0. (Buffers can be purchased from test kit supply companies, such as Hach or LaMotte.) Following are notes regarding buffers.

- The buffer solutions should be at room temperature when you calibrate the meter.
- Do not use a buffer after its expiration date.
- Always cap the buffers during storage to prevent contamination.
- Because buffer pH values change with temperature, the meter must have a built-in temperature sensor that automatically standardizes the pH when the meter is calibrated.
- Do not reuse buffer solutions!

TASK 3 Collect the sample

Refer to Task 2 in Chapter 5 - Water Quality Conditions for details on how to collect water samples using screw-cap bottles or Whirl-pak® bags.

TASK 4 Measure pH

The procedure for measuring pH is the same whether it is conducted in the field or lab.

If you are using a "pocket pal" or color comparator, follow the manufacturer's instructions. Use the following steps to determine the pH of your sample if you are using a meter.

1. Rinse the electrode well with deionized water.
2. Place the pH meter or electrode into the sample. Depress the dispenser button once to dispense electrolyte. Read and record the temperature and pH in the appropriate column on the data sheet. Rinse the electrode well with deionized water. 3. Measure the pH of the 4.01 and 7.0 buffers periodically to ensure that the meter is not drifting off calibration. If it has drifted, recalibrate it.

TASK 5 Return the field data sheets and samples to the lab or drop-off point.

Samples for pH must be analyzed within 2 hours of collection. If the samples cannot be analyzed in the field, keep the samples on ice and take them to the lab or drop-off point as soon as possible within the 2-hour limit.

References

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Data Form For Calculating Flow

DATA FORM FOR CALCULATING FLOW

Solving the equation: $\text{Flow} = \dfrac{A\,L\,C}{T}$

Where:

A = Average cross-sectional area of the stream. L = Length of the stream reach measured (usually 6.5 meters). C = A coefficient or correction factor (0.8 for rocky-bottom streams or 0.9 for muddy-bottom streams). T = Time, in seconds, for the float to travel the length of L.

A: Average Cross-Sectional Area

Transect #1 (upstream)

	Interval width (meters)	Depth (meters)	
A to B =	____	____	(at B)
B to C =	____	____	(at C)
C to D =	____	____	(at D)
D to E =	____	____	(shoreline)
Totals	[ ]	[ ]	÷ 4

= Avg. depth [ ] m

Cross-sectional area of Transect #1

= Total width (m) X Avg. depth (m)

[ ] X [ ] = [ ] m^2

Transect #2 (downstream)

	Interval width (meters)	Depth (meters)	
A to B =	____	____	(at B)
B to C =	____	____	(at C)
C to D =	____	____	(at D)
D to E =	____	____	(shoreline)
Totals	[ ]	[ ]	÷ 4

= Avg. depth [ ] m

Cross-sectional area of Transect #2

= Total width (m) X Avg. depth (m)

[ ] X [ ] = [ ] m^2

(Cross-sectional area of Transect #1 + Cross-sectional area of Transect #2) ÷ 2 = Average Cross-sectional area

A = ([ ] m^2 + [ ] m^2) ÷ 2 = [ ] m^2

L: Length of Stream Reach

[ ] m

C: Coefficient

[ ]

T: Travel Time

	Travel Time of Float (sec.)
Trial #1	____
Trial #2	____
Trial #3	____
Total	[ ] ÷ 3

= Avg. time [ ] sec.

Flow = $\dfrac{A\,L\,C}{T}$ = $\dfrac{[\ ]\ [\ ]\ [\ ]}{[\ ]}$ = [ ] **m^3/sec.**

Water Quality Assessment Data Sheet
2011-2012

Stream Name:	Site Code:
Latitude/Longitude:	Date/Time:
Site Description:	Investigators:

Weather conditions:

Now		Past 24 hours
___	Storm	___
___	Rain (steady)	___
___	Showers (intermittent)	___
___	Clear/sunny	___
___	% cloud cover	___

Has there been heavy rain in the last 7 days?
Air temperature (°C):
Comments: ____

Instream Features:

Parameter	Field Measurement
Water temperature	°C
Water pH	
Stream depth	m
Discharge (calculated on separate sheet)	m^3/s
Canopy cover	%
Obvious pollution	Yes or No Describe:

Comments:

Water Samples

Bacteria

What are fecal bacteria and why are they important?

Members of two bacteria groups, coliforms and fecal streptococci, are used as indicators of possible sewage contamination because they are commonly found in human and animal feces. The Streams Project tests coliforms. Although they are generally not harmful themselves, they indicate the possible presence of pathogenic (disease-causing) bacteria, viruses, and protozoans that also live in human and animal digestive systems. Therefore, their presence in streams suggests that pathogenic microorganisms might also be present and that swimming and eating shellfish might be a health risk. Since it is difficult, time-consuming, and expensive to test directly for the presence of a large variety of pathogens, water is usually tested for coliforms and fecal streptococci instead. Sources of fecal contamination to surface waters include wastewater treatment plants, on-site septic systems, domestic and wild animal manure, and storm runoff.

In addition to the possible health risk associated with the presence of elevated levels of fecal bacteria, they can also cause cloudy water, unpleasant odors, and an increased oxygen demand.

Indicator bacteria types and what they can tell you

The most commonly tested fecal bacteria indicators are total coliforms, fecal coliforms, *Escherichia coli*, fecal streptococci, and enterococci. All but *E. coli* are composed of a number of species of bacteria that share common characteristics such as shape, habitat, or behavior; *E. coli* is a single species in the fecal coliform group.

Total coliforms are a group of bacteria that are widespread in nature. All members of the total coliform group can occur in human feces, but some can also be present in animal manure, soil, and submerged wood and in other places outside the human body. Thus, the usefulness of total coliforms as an indicator of fecal contamination depends on the extent to which the bacteria species found are fecal and human in origin. For recreational waters, total coliforms are no longer recommended as an indicator. For drinking water, total coliforms are still the standard test because their presence indicates contamination of a water supply by an outside source.

Fecal coliforms, a subset of total coliform bacteria, are more fecal-specific in origin. However, even this group contains a genus, *Klebsiella*, with species that are not necessarily fecal in origin. *Klebsiella* are commonly associated with textile and pulp and paper mill wastes. Therefore, if these sources discharge to your stream, you might wish to consider monitoring more fecal and human-specific bacteria. For recreational waters, this group was the primary bacteria indicator until relatively recently, when EPA began recommending *E. coli* and enterococci as better indicators of health risk from water contact. Fecal coliforms are still being used in many states as the indicator bacteria.

E. coli is a species of fecal coliform bacteria that is specific to fecal material from humans and other warm-blooded animals. EPA recommends *E. coli* as the best indicator of health risk from water contact in recreational waters; some states have changed their water quality standards and are monitoring accordingly.

Which Bacteria Should You Monitor?

Which bacteria you test for depends on what you want to know. Do you want to know whether swimming in your stream poses a health risk? Do you want to know whether your stream is meeting state water quality standards?

Studies conducted by EPA to determine the correlation between different bacterial indicators and the occurrence of digestive system illness at swimming beaches suggest that the best indicators of health risk from recreational water contact in fresh water are *E. coli* and enterococci. For salt water, enterococci are the best. Interestingly, fecal coliforms as a group were determined to be a poor indicator of the risk of digestive system illness. However, many states continue to use fecal coliforms as their primary health risk indicator.

Sampling and equipment considerations

Bacteria can be difficult to sample and analyze, for many reasons. Natural bacteria levels in streams can vary significantly; bacteria conditions are strongly correlated with rainfall, and thus comparing wet and dry weather bacteria data can be a problem; many analytical methods have a low level of precision yet can be quite complex; and absolutely sterile conditions are required to collect and handle samples.

The primary equipment decision to make when sampling for bacteria is what type and size of sample container you will use. Once you have made that decision, the same, straightforward collection procedure is used regardless of the type of bacteria being monitored. Collection procedures are described under "How to Collect Samples" below.

It is critical when monitoring bacteria that all containers and surfaces with which the sample will come into contact be sterile. Containers made of either some form of plastic or Pyrex glass are acceptable to EPA. However, if the containers are to be reused, they must be sterilized using heat and pressure. The containers can be sterilized by using an autoclave, which is a machine that sterilizes containers with pressurized steam. If using an autoclave, the container material must be able to withstand high temperatures and pressure. Plastic containers either high-density polyethylene or polypropylene might be preferable to glass from a practical standpoint because they will better withstand breakage. In any case, be sure to check the manufacturer's specifications to see whether the container can withstand 15 minutes in an autoclave at a temperature of 121°C without melting. (Extreme caution is advised when working with an autoclave.) Disposable, sterile, plastic Whirl-pak® bags are used by a number of programs. The size of the container will depend on the sample amount

needed for the bacteria analysis method you choose and the amount needed for other analyses.

There are two basic methods for analyzing water samples for bacteria:

1. The membrane filtration method involves filtering several different-sized portions of the sample using filters with a standard diameter and pore size, placing each filter on a selective nutrient medium in a petri plate, incubating the plates at a specified temperature for a specified time period, and then counting the colonies that have grown on the filter. This method varies for different bacteria types (variations might include, for example, the nutrient medium type, the number and types of incubations, etc.).
2. **The multiple-tube fermentation method involves adding specified quantities of the sample to tubes containing a nutrient broth, incubating the tubes at a specified temperature for a specified time period, and then looking for the development of gas and/or turbidity that the bacteria produce. The presence or absence of gas in each tube is used to calculate an index known as the Most Probable Number (MPN). ← The Streams Project uses a variation of this method.**

Given the complexity of the analysis procedures and the equipment required, field analysis of bacteria is not recommended. Bacteria samples are sent to the UVM water quality lab for analysis.

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Phosphorus

Why is phosphorus important?

Both phosphorus and nitrogen are essential nutrients for the plants and animals that make up the aquatic food web. Since phosphorus is the nutrient in short supply in most fresh waters, even a modest increase in phosphorus can, under the right conditions, set off a whole chain of undesirable events in a stream including accelerated plant growth, algae blooms, low dissolved oxygen, and the death of certain fish, invertebrates, and other aquatic animals.

There are many sources of phosphorus, both natural and human. These include soil and rocks, wastewater treatment plants, runoff from fertilized lawns and cropland, failing septic systems, runoff from animal manure storage areas, disturbed land areas, drained wetlands, water treatment, and commercial cleaning preparations.

Forms of phosphorus

Phosphorus has a complicated story. Pure, "elemental" phosphorus (P) is rare. In nature, phosphorus usually exists as part of a phosphate molecule (PO_4). Phosphorus in aquatic systems occurs as organic phosphate and inorganic phosphate. Organic phosphate consists of a phosphate molecule associated with a carbon-based molecule, as in plant or animal tissue. Phosphate that is not associated with organic material is inorganic. Inorganic phosphorus is the form required by plants. Animals can use either organic or inorganic phosphate.

Both organic and inorganic phosphorus can either be dissolved in the water or suspended (attached to particles in the water column).

The phosphorus cycle

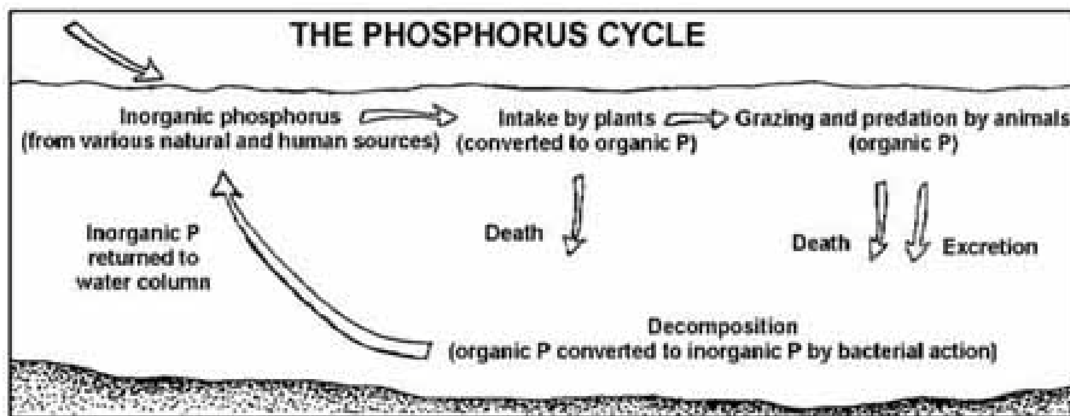

Figure 5.12

The phosphorus cycle
Phosphorus changes form as it cycles through the aquatic environment.

Phosphorus cycles through the environment, changing form as it does so (Fig. 5.12). Aquatic plants take in dissolved inorganic phosphorus and convert it to organic phosphorus as it becomes part of their tissues. Animals get the organic phosphorus they need by eating either aquatic plants, other animals, or decomposing plant and animal material.

As plants and animals excrete wastes or die, the organic phosphorus they contain sinks to the bottom, where bacterial decomposition converts it back to inorganic phosphorus, both dissolved and attached to particles. This inorganic phosphorus gets back into the water column when the bottom is stirred up by animals, human activity, chemical interactions, or water currents. Then it is taken up by plants and the cycle begins again.

In a stream system, the phosphorus cycle tends to move phosphorus downstream as the current carries decomposing plant and animal tissue and dissolved phosphorus. It becomes stationary only when it is taken up by plants or is bound to particles that settle to the bottom of pools.

In the field of water quality chemistry, phosphorus is described using several terms. Some of these terms are chemistry based (referring to chemically based compounds), and others are methods-based (they describe what is measured by a particular method).

The term "orthophosphate" is a chemistry-based term that refers to the phosphate molecule all by itself. "Reactive phosphorus" is a corresponding method-based term that describes what you are actually measuring when you perform the test for orthophosphate. Because the lab procedure isn't quite perfect, you get mostly orthophosphate but you also get a small fraction of some other forms.

More complex inorganic phosphate compounds are referred to as "condensed phosphates" or "polyphosphates." The method-based term for these forms is "acid hydrolyzable."

Monitoring phosphorus

Monitoring phosphorus is challenging because it involves measuring very low concentrations down to 0.01 milligram per liter (mg/L) or even lower. Even such very low concentrations of phosphorus can have a dramatic impact on streams. Less sensitive methods should be used only to identify serious problem areas.

While there are many tests for phosphorus, only four are likely to be performed by volunteer monitors.

1. The *total orthophosphate* test is largely a measure of orthophosphate. Because the sample is not filtered, the procedure measures both dissolved and suspended orthophosphate. The EPA-approved method for measuring total orthophosphate is known as the ascorbic acid method. Briefly, a reagent (either liquid or powder) containing ascorbic acid and ammonium molybdate reacts with orthophosphate in the sample to form a blue compound. The intensity of the blue color is directly proportional to the amount of orthophosphate in the water.
2. **The *total phosphorus* test measures all the forms of phosphorus in the sample (orthophosphate, condensed phosphate, and organic phosphate). This is accomplished by first "digesting" (heating and acidifying) the sample to convert all the other forms to orthophosphate. Then the orthophosphate is measured by the ascorbic acid method. Because the sample is not filtered, the procedure measures**

both dissolved and suspended orthophosphate. ← The <u>Streams Project</u> uses this method.

3. The *dissolved phosphorus* test measures that fraction of the total phosphorus which is in solution in the water (as opposed to being attached to suspended particles). It is determined by first filtering the sample, then analyzing the filtered sample for total phosphorus.
4. *Insoluble phosphorus* is calculated by subtracting the dissolved phosphorus result from the total phosphorus result.

All these tests have one thing in common they all depend on measuring orthophosphate. The total orthophosphate test measures the orthophosphate that is already present in the sample. The others measure that which is already present and that which is formed when the other forms of phosphorus are converted to orthophosphate by digestion.

Sampling and equipment considerations

Monitoring phosphorus involves two basic steps:

- Collecting a water sample
- Analyzing it in the field or lab for one of the types of phosphorus described above. The laboratory analysis method/protocols are included at the end of this section.

Sample Containers

Sample containers made of either some form of plastic or Pyrex glass are acceptable to EPA. Because phosphorus molecules have a tendency to "adsorb" (attach) to the inside surface of sample containers, if containers are to be reused they must be acid-washed to remove adsorbed phosphorus. Therefore, the container must be able to withstand repeated contact with hydrochloric acid. Plastic containers either high-density polyethylene or polypropylene might be preferable to glass from a practical standpoint because they will better withstand breakage. Some programs use disposable, sterile, plastic Whirl-pak® bags. The size of the container will depend on the sample amount needed for the phosphorus analysis method you choose and the amount needed for other analyses you intend to perform.

Dedicated Labware

All containers that will hold water samples or come into contact with reagents used in this test must be dedicated. That is, they should not be used for other tests. This is to eliminate the possibility that reagents containing phosphorus will contaminate the labware. All labware and containers that contain water for phosphorus analysis should be acid-washed. The only form of phosphorus this manual recommends for field analysis is total orthophosphate, which uses the ascorbic acid method on an untreated sample. Analysis of any of the other forms requires adding potentially hazardous reagents, heating the sample to boiling, and using too much time and too much equipment to be practical. In addition, analysis for other forms of phosphorus is prone to errors and inaccuracies in a field situation. Pretreatment and analysis for these other forms should be handled in a laboratory.

Ascorbic Acid Method

In the ascorbic acid method, a mix of reagents including sulfuric acid, potassium antimonyl tartrate, ammonium molybdate, and ascorbic acid added to the water sample. This colors the sample blue in direct proportion to the amount of orthophosphate in the sample. A spectrophotometer, a specialized laboratory instrument, measures the amount of light absorbed or transmitted at a wavelength of 700 - 880 nanometers. This measurement is called “absorbance” or “transmittance”.

We translate that absorbance into a meaningful phosphorus concentration (mg/L) by referencing a standard curve. The standard curve is a series of standard solutions with known concentrations of phosphorus. Those standards are the basis of our standard curve, which provides us with a reference for measuring unknown samples collected from the field.

References

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Most of this information was retrieved in May 2010 from: http://www.epa.gov/volunteer/stream/index.html However, some of this information was edited by Streams Project staff.

Total Suspended Solids (TSS)

What are total solids and why are they important?

Total solids are dissolved solids plus suspended and settleable solids in water. In stream water, dissolved solids consist of calcium, chlorides, nitrate, phosphorus, iron, sulfur, and other ions particles that will pass through a filter with pores of around 2 microns (0.002 cm) in size. Suspended solids include silt and clay particles, plankton, algae, fine organic debris, and other particulate matter. These are particles that will not pass through a 2-micron filter.

The concentration of total dissolved solids affects the water balance in the cells of aquatic organisms. An organism placed in water with a very low level of solids, such as distilled water, will swell up because water will tend to move into its cells, which have a higher concentration of solids. An organism placed in water with a high concentration of solids will shrink somewhat because the water in its cells will tend to move out. This will in turn affect the organism's ability to maintain the proper cell density, making it difficult to keep its position in the water column. It might float up or sink down to a depth to which it is not adapted, and it might not survive.

Higher concentrations of suspended solids can serve as carriers of toxics, which readily cling to suspended particles. This is particularly a concern where pesticides are being used on irrigated crops. Where solids are high, pesticide concentrations may increase well beyond those of the original application as the irrigation water travels down irrigation ditches. Higher levels of solids can also clog irrigation devices and might become so high that irrigated plant roots will lose water rather than gain it.

A high concentration of total solids will make drinking water unpalatable and might have an adverse effect on people who are not used to drinking such water. Levels of total solids that are too high or too low can also reduce the efficiency of wastewater treatment plants, as well as the operation of industrial processes that use raw water.

Total solids also affect water clarity. Higher solids decrease the passage of light through water, thereby slowing photosynthesis by aquatic plants. Water will heat up more rapidly and hold more heat; this, in turn, might adversely affect aquatic life that has adapted to a lower temperature regime.

Sources of total solids include industrial discharges, sewage, fertilizers, road runoff, and soil erosion. Total solids are measured in milligrams per liter (mg/L).

Sampling and equipment considerations

Total solids are important to measure in areas where there are discharges from sewage treatment plants, industrial plants, or extensive crop irrigation. In particular, streams and rivers in arid regions where water is scarce and evaporation is high tend to have higher concentrations of solids and are more readily affected by human introduction of solids from land use activities.
Total solids measurements can be useful as an indicator of the effects of runoff from construction, agricultural practices, logging activities, sewage treatment plant discharges, and other sources. **As with turbidity, concentrations often increase sharply during rainfall, especially in developed watersheds**. They can also rise sharply during dry weather if earth-disturbing activities are occurring in or near the stream without erosion control practices in place. Regular monitoring of total solids can help detect trends that might indicate increasing erosion in developing watersheds. Total solids are related closely to stream flow and velocity and should be correlated with these factors. Any change in total solids over time should be measured at the same site at the same flow.

Summary: How does the Streams Project measure Total Suspended Solids (TSS)?

Total solids are measured by weighing the amount of solids present in a known volume of sample. This is done by weighing a beaker, filling it with a known volume, evaporating the water in an oven and completely drying the residue, and then weighing the beaker with the residue. The total solids concentration is equal to the difference between the weight of the beaker with the residue and the weight of the beaker without it. Since the residue is so light in weight, the lab will need a balance that is sensitive to weights in the range of 0.0001 gram. Balances of this type are called analytical or Mettler balances, and they are expensive (around $3,000). The technique requires that the beakers be kept in a desiccator, which is a sealed glass container that contains material that absorbs moisture and ensures that the weighing is not biased by water condensing on the beaker. Some desiccants change color to indicate moisture content.

The measurement of total solids cannot be done in the field. Samples must be collected using clean glass or plastic bottles or Whirl-pak® bags and taken to a laboratory where the test can be run.

References

APHA. 1992. *Standard methods for the examination of water and wastewater.* 18th ed. American Public Health Association, Washington, DC.

Field Method: Water Samples for Bacteria, TSS & Phosphorus

I. Introduction

Water quality samples are taken for total phosphorus, total suspended solids, and *Escherichia coli* at two stream sites biweekly. Samples are collected in analyte-specific screw-top sample bottles. Following collection, the samples are kept cold and delivered to the Water Quality Laboratory for analysis.

II. Equipment and Materials

a. Waders
b. Labels and Permanent Marker
c. Bottle of sterile E. coli blank water
d. Bottle Phosphorus blank water
e. Gloves
f. Cooler
g. Ice packs or ice
h. Sample bottles: 4 Total Phosphorus bottles per site (8 total), 4 *E. coli* bottles per site (8 total), and 3 TSS bottles per site (6 total)

III. Prior to Departure

a. Label all bottles according to the Creating Field Labels protocol with the site code, date, analyte and replicate number

b. Confirm that you have all the equipment and materials listed above

IV. Prior to Sample Collection: Field Blanks

a. Before taking stream samplings, field blanks must be taken for each set of total phosphorus and *E. coli* samples.

b. Wearing gloves, pour total phosphorus blank water into the phosphorus blank bottle, leaving approximately 1 inch of air space. See Figures 1 and 2.

Figure 1

Figure 2

c. Wearing gloves, pour *E. coli* blank water into the E. coli blank bottle, making sure the level is above the Fill Line. See Figure 3 and 4.

Figure 3

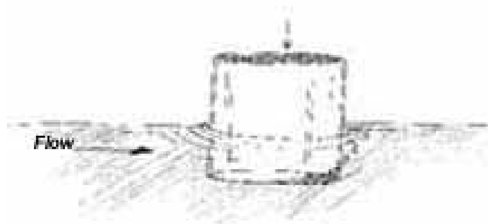
Figure 4

Sample Collection

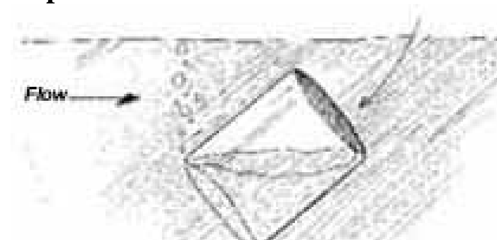
d. Enter the water downstream from your sampling site and move away from the stream bank into the main current. Avoid disturbing the sediment as much as possible.
e. Stand facing upstream.

f. Wearing gloves, remove the cap from the bottle just before sampling. Avoid touching the inside of the bottle or cap. If you accidentally touch the inside of the bottle, use another one.
g. Hold the bottle near its base and plunge it (opening downward) below the water surface. Collect a water sample 8 to 12 inches beneath the surface or mid-way between the surface and the bottom if the stream reach is shallow.
h. Turn the bottle underwater into the current. In slow-moving stream reaches, push the bottle underneath the surface and in the upstream direction.

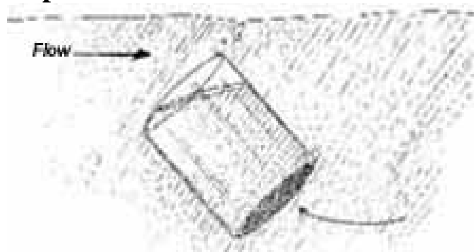
Step 1



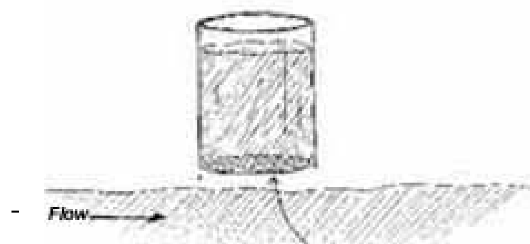
Step 2



Step 3



Step 4



i. If necessary, adjust the volume of sample by gently pouring water out of the bottle
    i. Total Phosphorus samples: see Figure 2 for filling directions
    ii. E. coli samples: see Figure 4 for filling directions
    iii. TSS samples: fill completely
j. Make sure to complete the above steps (in Section V) for three total phosphorus, *E. coli*, and TSS bottles at each site.
k. Place all samples, including blanks, in a cooler on ice for transport back to school or to the Water Quality Laboratory

Figure 6

Water Quality Field Labels

*Please use labels provided to you by the Streams staff

Site Code Date Analyte and Replicate Number

Analyte Abbreviations:
Phosphorus: **TP**
E. coli: **EC**
Total Suspended Solids: **TSS**

E. coli Samples

WR_CeBrk_259
4/26/2010
EC 1 of 3

Total Phosphorus Samples

WR_CeBrk_259
4/26/2010
TP 1 of 3

Total Suspended Solids

WR_CeBrk_259
4/26/2010
TSS 1 of 3

Water Quality Laboratory Methods

Lab Method: Determination of Bacteria Using IDEXX Colilert System

I. Materials

a. Colilert growth media packets
b. Quanti trays (enough for samples, dilutions)
c. Pipetters & pipet tips
d. Extra 120 mL sterile bottles for dilutions
e. Quanti-tray sealer
f. Incubator at 35 °C
g. UV viewing light
h. MPN chart
i. Ethyl Alcohol (75%) for cleaning
j. Data book

II. Protocol

a. Turn on Quanti Tray sealer. Green light must be on before running any trays through the machine.
b. Collect all necessary materials (listed above) to do analysis.
c. Only process 2-3 samples at one time. Leave remaining in the fridge and retrieve as needed.
d Assess whether a dilution will be needed for each sample (is sample highly turbid, are there large particulates floating on the surface?)
e. Prepare sample dilutions, see table below for proper volume ratios:

Dilution	Sterilized H_2O	Sample
1:1	0	100 mL
1:10	90 mL	10 mL
1:100	99 mL	1 mL
1:1000	99.9 mL	100 mL
1:10,000	99.99 mL	10 mL

f. If no dilution needed, be sure that sample bottle contains only 100 mL (pour out additional amount of sample if above fill line).
g. Add 1 packet of Colilert media to each 100 mL sample. Shake bottle and allow media to completely dissolve.
h. Pour 100 mL sample into Quanti Tray. Make sure green light is illuminated on the Quanti-Tray sealer.
i. Place tray face-down on rubber tray holder. Send tray through Quanti-tray sealer. Check to be sure that tray is properly sealed.
j. Place tray into incubator at 35 °C. Record time tray was placed into oven and leave tray to incubate for 24 hours (± 30 minutes).
k. Retrieve tray from oven after 24 hour incubation and count number of cells that have changed color in regular light (total coliform) and under UV lamp (E. coli). Record on datasheet.
l. Using MPN chart, determine number of total coliform and E. coli bacteria present in each sample. Record on datasheet and store in E. coli data folder.

Lab Method: Determination of Total Suspended Solids (TSS)

I. **Introduction**

This method for the determination of total suspended solids (TSS) is borrowed from Standard Methods for the Examination of Water & Wastewater (APHA 2005) and Wetzel & Likens 2000. A well-shaken stream sample is filtered through a weighted filter and residue retained on the filter is dried at 103 to 105°C. The increase in weight of the filter is equivalent to the total suspended solids in the stream sample.

II. **Equipment and Materials**

a. Analytical balance
b. 1L filter flask
c. Vacuum pump
d. Vacuum tubing
e. Aluminum trays
f. 47mm glass-fiber filters
g. Filter tower
h. Graduated cylinder
i. Forceps
j. Spatula

III. **Procedure**

a. Dry filters
    i. Add new glass-fiber filters to clean aluminum trays
    ii. Dry the filters in the drying oven at 103-105°C for a minimum of two hours
b. Remove TSS samples from the refrigerator and record the sample codes on the TSS Data Entry Form
c. Remove the filters from the drying oven and label the aluminum trays with the sample codes
d. Determine the Pre-Drying filter weight
    i. Using tweezers, remove the filter from the aluminum tray
    ii. Weigh the aluminum tray alone and record as the Pan Weight (g) on the TSS Data Entry Form
    iii. Weigh the filter alone and record as the Pre-Drying Filter Weight (g)
    iv. Return the filter to the aluminum tray
e. Set up filter apparatus
    i. Connect the vacuum flask to the filter flask with thevacuum tubing
    ii. Insert the magnetic base of the filter tower into the top of the filter flask
f. Filter sample
    i. Using tweezers, place a dried and weighed filter on top of the filter base. Make sure it is centered and the ends are not hanging over the edge
    ii. Place the top of the filter tower on top of its base. The magnet may cause the filter to move, so make sure that the filter is still centered
    iii. Shake the stream sample to suspend any solids present in the sample
    iv. Pour the sample into a graduated cylinder and record the sample volume as the Volume of Sample Filtered (mL)on the TSS Data Entry Form
    v. Turn on the vacuum pump and slowly pour the sample into the filter tower, making sure that it does not overflow
    vi. Allow all the water to be pulled through the filter. Continue suction to allow complete drainage of the water

vii. Turn off the vacuum pump. Gently lift the top of the filter tower off of its base, being sure not to tear to the filter

viii. Using the spatula, gently remove the filter from the base and place in the designated aluminum tray

ix. Dry the filter in a drying oven at 103-105°C for a minimum of two hours

g. Determine final weight

i. Remove dried filter from the oven

ii. Weight the filter and the aluminum tray together and record as the Post-Drying Filter Weight (g)

Lab Method: Determination of Total Phosphorus

Introduction

This method for the determination of total phosphorus (TP) is borrowed from Standard Methods for the Examination of Water & Wastewater (APHA 2005) and Wetzel & Likens 2000. The sample is digested in an acidic ammonium persulfate solution, reducing all forms of phosphorus into orthophosphate. The digested sample is then treated with a “combined reagent” consisting of ammonium molybdate, potassium antimonyl tartrate and ascorbic acid. The ammonium molybdate and potassium antimonyl tartrate react in an acidic medium with orthophosphate to form a heteropoly acid, phosphomolybdic acid. This compound is then reduced to a blue colored solution by ascorbic acid, and analyzed colorimetrically at 885 nm. This method can be used after various digestions with acid (e.g., persulfate digestion) to determine the concentrations of total dissolved phosphorus (TDP) and total particulate phorphorus (TP) (Standard Methods).

I. Preparations and Precautions

a. Sign up for the autoclave (Marsh Life Science, 1st or 3rd floor) prior to beginning this procedure. Be sure to enter the time in the notebook and on the whiteboard.
b. All reagents and standards should be made and stored in acid washed glassware and containers.
c. All wastes from this test should be saved and stored in designated hazardous waste containers according the Hazardous Waste Disposal Protocol. Ensure that space is available in waste containers before beginning the analysis.

II. Personal Protective Equipment (PPE):

a. Goggles
b. Lab coat
c. Closed-toe shoes
d. Purple nitrile gloves

Digestion Procedure

I. Materials and Equipment

a. 125mL Erlenmeyer flasks
b. Volumetric flasks: 1L, 250mL, 100mL
c. Graduated cylinders: 50mL and 100mL
d. 1L media bottles
e. Pipetters: 1-5mL, 100-1000µL, 20-200µL
f. Aluminum foil
g. Autoclavable plastic bins
h. Autoclave and autoclave gloves

II. Preparation of Standards and Samples

a. Prepare Digestion Mix

i. <u>Make 5.6M sulfuric acid</u>: Add 72 mL concentrated sulfuric acid to 178mL HPLC water. The solution will get hot! Swirl and cool in cold water bath or in hood. *Store in the secondary container labeled Inorganic Acid Solutions in the corrosives cabinet under the hood.*
ii. <u>Make Ammonium Persulfate solution</u>: Dissolve 20g of ammonium persulfate in 100mL of HPLC water in a volumetric flask. *Make fresh for every run.*

iii. Make Digestion Mix: Combine 2 parts ammonium persulfate solution with 1 part 5.6M sulfuric acid. Prepare in excess. See the following example:

1. Determine total volume of digestion mix required:

   18 standards x 3.0mL = 54mL
   30 samples x 3.0mL = 90 mL
   144mL

   144mL of digestion mix is required for this example. Therefore, prepare 150mL for this example

   - 100mL ammonium persulfate
   - 50mL 5.6M sulfuric acid

b. Preparing Standards

i. Make Working Stock (2.5 µg/mL phosphorus stock solution): Dilute 50.0 mL of standard phosphorus solution (Fisher #58304, 50 µg/mL) to 1000mL with HPLC water in volumetric flask (or 25 mL to 500mL). Store in the refrigerator.

ii. Prepare standard dilutions with a total volume of 50mL in duplicate from the Working Stock solution according to Table 1. Label each flask with permanent marker.

Table 1. Example dilution of stock solution for analysis standards.

Concentration of working stock = 2.5 µg/mL							
Standard concentration	**0**	**5**	**25**	**50**	**75**	**100**	**200**
µl Working stock	0	100	500	1000	1500	2000	4000
ml HPLC water	50	49.9	49.5	49	48.5	48	46

iii. Add 3.0mL of digestion mix to each 50mL sample.

iv. Cover each flask with aluminum foil to minimize volume loss and contamination while transporting and autoclaving.

c. Preparing QC Standards

i. Dry potassium dihydrogen phosphate (KH_2PO_4) 24 hours at 105˚C in the drying oven.

ii. Make QC Stock (20mg/L PO_4^{-3} stock solution): Dilute 136.1mg of dried KH_2PO_4 with HPLC water to 1000mL in volumetric flask. Store in the refrigerator.

iii. Prepare dilutions with concentrations of 0µg/L, 30µg/L, and 60µg/L in duplicate from the QC Stock solution according to Table 2. A dilution of 90µg/L should also be prepared if sample concentrations are anticipated to measure consistently above 100µg/L. Label each flask with permanent marker.

Table 2. Dilution of stock solution for QC samples

iv. **QC Concentration (µg/L)**	**0**	**30**	**60**	**90**
v. µL Stock Solution	0	50	100	150
vi. mL HPLC Water	50	49.95	49.9	49.85

vii. Add 3.0mL of digestion mix to each 50mL sample

viii. Cover each flask with aluminum foil to minimize volume loss and contamination while transporting and autoclaving

d. Preparing Samples

i. Thaw samples

1. Samples may be removed from the freezer and left to thaw at room temperature the night before analysis
2. OR, samples may be thawed in a warm water bath the morning of analysis

ii. Shake sample bottle to thoroughly mix.
iii. Measure out 50mL of completely thawed, room temperature sample and pour into a labeled 125mL flask.
iv. Prepare approximately every 10th sample in duplicate.
v. Add 3.0mL of digestion mix to each 50mL sample
vi. Cover each flask with aluminum foil to minimize volume loss and contamination while transporting and autoclaving
vii. Return the sample bottles to the freezer.

III. Autoclave Digestion

a. Sign up for a one-hour time slot in the autoclave prior to analysis. The schedule can sometimes get pretty busy.
b. Prepare samples to be transported by cart to Marsh Life Sciences:
   i. Be sure that the flasks are tightly covered with aluminum foil.
   ii. Place flasks in small, divided boxes
   iii. Be sure that you have two autoclaveable bins.
   iv. Transport the samples via cart.
c. Autoclave all samples at 121°C for 30 minutes.
   i. Make sure the autoclave has finished its previous cycle and fully depressurized. DO NOT open the door if the pressure is above 0! This is read on the gauge on the front of the autoclave.
   ii. Transfer samples from boxes to the plastic bins. NOTE: Samples and containers MUST be in a secondary container inside the autoclave.
   iii. Place bins inside the autoclave and close the door tightly.
   iv. Behind the plastic window, set the autoclave cycle to 30 minutes Liquid, 0 minutes Dry. Press the large square "Liquid" button.
   v. If the small green light is on, press the "Reset" button.
   vi. Press On/Start.
   vii. The entire cycle usually takes ~45 minutes, including start-up and cool-down times.
d. Wearing autoclave gloves, remove the bins from the autoclave. CAUTION: steam and samples will be very hot.
e. Still wearing gloves, transfer samples from bins to the boxes and transport back to Cook

IV. Post-Digestion

a. Remove samples from cardboard boxes
b. Allow to cool to room temperature.
c. If samples are "chunky" (large amount of sediment or debris present):
   i. **Post-Digestion Filtering**
      1. Use 0.45 um disposable glass microfiber filters.
      2. Use a few drops of sample to wet filter.
      3. Filter digested sample into a new 125mL flask
d. NOTE: It is safe to store digested samples in the refrigerator up to 2 days before analysis on the spectrophotometer. There was no significant difference between samples run immediately following digestion and after 2 days of storage.

Ascorbic Acid Method Analysis on Spectrophotometer

I. **Materials and Equipment**
   a. All digested samples, standards, and QC standards
   b. 60mL syringe with attached tubing
   c. 100mL graduated cylinder
   d. Pipetter and pipette tips: 10-100 ☐L, 100-1000 ☐L, 1-5mL
   e. Titration Set-Up: Ring stand, clamps, 25mL glass burette, tray
   f. Spectrophotometer with 2 10cm flowpath cuvettes
   g. 1L Suction flask with vacuum attachment and tubing
   h. Vacuum pump
   i. Analytical Balance
   j. Computer with UVProbe Software
   k. Laboratory Total Phosphorus Data Entry Logbook
   l. Waste Accumulation Container

II. **Preparation of 1N Sodium Hydroxide**
   a. Weigh out 40g Sodium Hydroxide pellets on the analytical balance (due to the pellets, it will never reach exactly 40g. Value should be within ±0.1g
   b. In a 1L media bottle, dissolve the sodium hydroxide in 1000mL HPLC water
   c. Allow the pellets to dissolve.
   d. Shake well before use

III. **Preparation of Combined Reagent** *(Make less than 30 minutes before use)*
   a. Prepare the following reagents:
      i. Ammonium Molybdate solution: dissolve 3.0g [(NH4) 6Mo7O244H20] in HPLC water and dilute to 100 mL in volumetric flask. *Store in fridge. Discard after 6 months*.
      ii. 15 % Sulfuric Acid: Under a hood, add 30 mL of concentrated sulfuric acid to 170 mL of HPLC water in an Erlenmeyer flask. Store in acid-proof container in corrosives cabinet.
      iii. Ascorbic acid solution: dissolve 5.4g of L-ascorbic acid in HPLC water and dilute to 100mL. NOTE: Ascorbic acid must be stored frozen or made fresh, if frozen make sure that it is completely thawed before use. Immediately return to fridge or freezer after use. *Solution is stable for 1 week at 4°C in the refrigerator*
      iv. Potassium antimonyl-tartrate solution: dissolve 0.14g of [$K(SbO)CH_4O_6$ $0.5H_2O$] in HPLC water and dilute to 100mL. *Store in glass container in fridge. Discard after 2 months.*
   b. Combine the reagents in the order listed and in the ratios shown in Table 3 .
      i. The combined reagent is light-sensitive. Prepare in an amber bottle.
      ii. DO NOT STORE LONGER THAN 6 HOURS.
      iii. Prepare enough volume to add 3.0mL of combined reagent to each standard and unknown.

Table 3. Add the components in the order listed and in the 2:5:2:1 ratio shown

Total Volume Desired (mL)	Ammonium Molybdate (mL)	Sulfuric acid (mL)	Ascorbic acid (mL)	Potassium Antimonyl Tartrate (mL)
10	2.0	5.0	2.0	1.0
15	3.0	7.5	3.0	1.5
25	5.0	12.5	5.0	2.5
50	10.0	25.0	10.0	5.0
100	20.0	50.0	20.0	10.0
120	24.0	60.0	24.0	12.0
140	28.0	70.0	28.0	14.0
150	30.0	75.0	30.0	15.0
200	40.0	100.0	40.0	20.0
300	60.0	150.0	60.0	30.0
750	150.0	375.0	150.0	75.0

I. Sample Analysis Procedure

a. While samples are cooling, prepare the combined reagent as described above.

b. When cool, add 1 drop (~20µL) of phenolphthalein to each sample.

c. Titrate to neutral pH, as indicated by a light pink color, with 1N Sodium Hydroxide solution. Record the volume added to each flask in Total Phosphorus Data Entry book.

d. Pour 30mL of the titrated sample into a labeled 50mL centrifuge tube (measure by graduations on tube)

e. Immediately pipette 3.0mL of combined reagent to sample centrifuge tube. Mix by inverting the capped tube. Record the Time Added in the notebook.

f. Allow samples to incubate no less than 10 minutes and no more 1 hour prior to measuring the absorbance

g. Read the absorbance of the samples on the UV-1800 spectrophotometer at 885nm using a 10 cm flowpath cell.

   i. Start up spectrophotometer and prepare the UVProbe software file as described in the UV-1800 Operation Protocol

   ii. Following the completion of the Start Procedure, remove the HPLC water from the front cuvette using the vacuum apparatus

   iii. Rinse the cuvette twice with HPLC water using the syringe with attached tubing

   iv. Starting with the first standard, add 30mL of the incubated sample to the front cuvette with the syringe. Make sure there are no bubbles. Close with white plugs.

   v. Run the standards and samples according to the Analysis Procedure of the UV-1800 Operation Protocol.

vi. When absorbance has been determined, remove the sample from the cuvette with the vacuum apparatus. Rinse the cuvette with HPLC water every 5 samples and after high absorbance standards and samples

h. After all samples have been analyzed, save the results on the computer
i. Shut down and clean up the spectrophotometer according to the Shut Down Procedure of the UV-1800 Operation Protocol
j. Record the r-squared value and the absorbance and concentration of each sample in the notebook.

II. Waste Disposal and Clean up

a. Excess volumes of neutralized sample that was left over in the flasks following titration and DOES NOT CONTAIN COMBINED REAGENT may be dumped down the drain.
b. Excess volumes of sample in the centrifuge tubes containing combined reagent must be disposed of into labeled waste containers
c. Dispose of waste accumulated in the suction flask into labeled waste containers
d. Rinse sample tubes, caps, reagent bottle, syringe, and cuvette twice with tap water into the waste containers
e. Remove all permanent marker with ethanol
f. Acid wash dirty glassware.

Literature Cited

American Public Health Association. 2005. Standard Methods for the Examination of Water and Wastewater. American Public Health Association. Washington, D.C.

Wetzel, R.G. and G.E. Likens. 2000. Limnological Analyses, 3rd Ed. Springer-Verlag. New York, NY.

Section 4: Macroinvertebrates

Contents

Macroinvertebrates General Info 2

Macroinvertebrate Equipment Field Checklist 3

Macroinvertebrate Communities in Wadeable Streams 4

Field Methods: Macroinvertebrate Sampling 5

Field Method: Modified Pebble Count of Riffle Habitat 6

Laboratory Method: Identifying, Preserving, & Counting Macroinverts 7

Macroinvertebrate Field Labels 8

Benthic Macrointerbrate Habitat Data Sheet 9

Macroinvertebrate Data Sheet 10

Macroinvertebrates General Info

There are several components to assessing macroinvertebrate communities in streams:

- Discharge or flow
- Substrate
- Macroinvertebrate Identification

Undergraduates: Collect macroinvertebrate samples according to research plan developed with your mentor. Note: Not all undergraduates will collect macroinvertebrate samples, but all undergraduates should understand how they are collected and that they can be used as biological indicators of water quality. Complete a "WATER QUALITY ASSESTMENT DATA SHEET" each time you visit and sample the stream.

High School Teams: Take all the components listed above each time you sample for macroinvertebrates. You should sample for macroinvertebrates once during the school year. Use the "BENTHIC MACROINVERTEBRAT DATA SHEET" and the "MACROINVERTEBRATE DATA SHEET". Also, complete the "WATER QUALITY ASSESTMENT DATA SHEET" each time you visit and sample the stream.

All Participants: The data sheet and field labels that you need to take macroinvertebrate samples are at the end of this section and there are extra copies in the "Extra Data Sheets" section of your manual. You should enter the data that you collect and record in the field on your "Water Quality Assessment Data Sheet" online as soon as possible after your field visit.

Macroinvertebrate Equipment Field Checklist

General Equipment

- Waders
- Spray bottles with disinfectant solution and water
- Gloves
- Pencils and permanent marker
- Datasheets (one for each site)
- Clipboard
- Insect repellent/sunscreen
- Sunglasses
- Camera
- First Aid kit
- Cell phone

Physical parameters

- Meter tape
- Meter stick
- Water temperature/pH meter
- Stopwatch
- Tennis ball
- Gravelometer

Bug collection

- Kick net
- Kitty litter tray
- #30 sieve
- 4 Whirlpaks for each site + extras
- 2 large mason jars per site
- Ethanol
- Field forceps
- Plastic spoon

Macroinvertebrate Communities in Wadeable Streams

All macroinvertebrate samples are collected during the late-Summer, early-fall index period, from September to mid-October unless otherwise discussed. A field crew selects a representative riffle section in the steam reach to be sampled. Physical characteristics recorded at the selected each site include: stream width, depth, water velocity, water temperature, weather conditions, substrate composition, substrate embeddedness (riffles sites only), canopy cover, stream bank condition, and immediate upstream land use. All data are entered onto a field sheet with appropriate site and sampling event identifiers, along with additional comments that may be applicable to the site evaluation. This sampling protocol is based on methods used by the Vermont Department of Environmental Conservation.

Field Methods: Macroinvertebrate Sampling

Samples are collected using an 18 inch wide x 9 inch high rectangular frame net with a 500 micron mesh size.

1) One person operates the net while the other person operates the stopwatch.

2) Place the net in the riffle, being sure the base of the net is firmly set against the stream bottom and there is water flowing into the net.

3) Using your hands, disturb an area immediately upstream of the net (square area, 18" x 18"), ensuring that all pieces of substrate are moved and rubbed clean of attached organisms and flow into the net opening. After scrubbing the larger substrates, disturb any underlying gravel to an approximate depth of 10 cm. This typically takes about 30 seconds but it is more important to complete the procedure than to exactly time 30 seconds.

4) Turn the contents of the net inside out into the kitty litter tray with lots of rinse water taken from the stream.

5) Rinse and scrub large gravel of remaining organisms and remove it from the net along with leaves and sticks. Any material adhered to gravel, leaves, and sticks is likely to contain macroinvertebrates, so be thorough.

6) Transfer the contents of the kitty litter tray into a #30 (=600 μm) sieve to remove small particles and water from the sample.

7) Using forceps and a plastic spoon, transfer contents of sieve into a Whirl pak ® and fill approximately half the Whirl pak® with 100% ethanol, being sure to cover the entire sample but also leave enough room to close the bag. Be sure the Whirl pak ® contains a paper label with the following information: Stream name @ road name, town/state/country, latitude/longitude, month-day-year, Collector name/School name, sample code (080624-010,020 etc). The label should be inside the bag in the ethanol – for that reason, do not use pen or ink-jet printouts. If a sample occupies more than one bag then label each part of the sample with the same sample number and write *1 of 3; 2 of 3…* etc. <u>Do not rely on sharpie markings on the outside of the bag. Leaking ethanol removes all traces and the sample becomes useless</u>.

8) Turn the net inside out and rinse thoroughly to remove debris. Use the net inside out for the next sample. The act of sampling will further rinse the outside of the net (and it will become the inside for the following sample).

9) Moving up-stream, repeat steps 1-8 at 3 additional locations within the riffle *representing a range of velocities and substrate types* characteristic of that riffle, being careful to avoid areas that have been previously disturbed. The total active sampling time should roughly equal 2 minutes for the sampling site (approximately 30 seconds at each location). Do not mix the four samples – they should be maintained as separate samples through all field and lab procedures.

10) You will end up with 4 separate samples from each site. Store 4 Whirl-paks in quart-size mason jar (or as many as are needed) until ready to process. This "composite" sampling methodology effectively collects samples representative of the entire macroinvertebrate community of that riffle.

Field Method: Modified Pebble Count of Riffle Habitat

This method is used to describe the substrate particle size classes within the “riffle” habitat of high gradient stream types that is targeted by the VTDEC for macroinvertebrate community assessments. The method is based on the more rigorous technigue developed by Wolmen (1954) to describe coarse river bed materials, and modifications of this technique developed by the Forest Service developed to describe the channel bed materials within stream reaches Bevenger and King (1995) and Harrelson, et al (199?).

Riffle Pebble count Procedure:
1. A minimum of 100 particles are to be recorded on a tally sheet.

2. Diagonal transects across the stream are paced off until a minimum 100 count is reached. Transects begin at the lower end of the wetted portion of the stream bed within the macroinvertebrate sampling section or riffle. A pebble is selected as described below every two paces in larger streams > 20m across, or every pace in smaller streams <20m across.

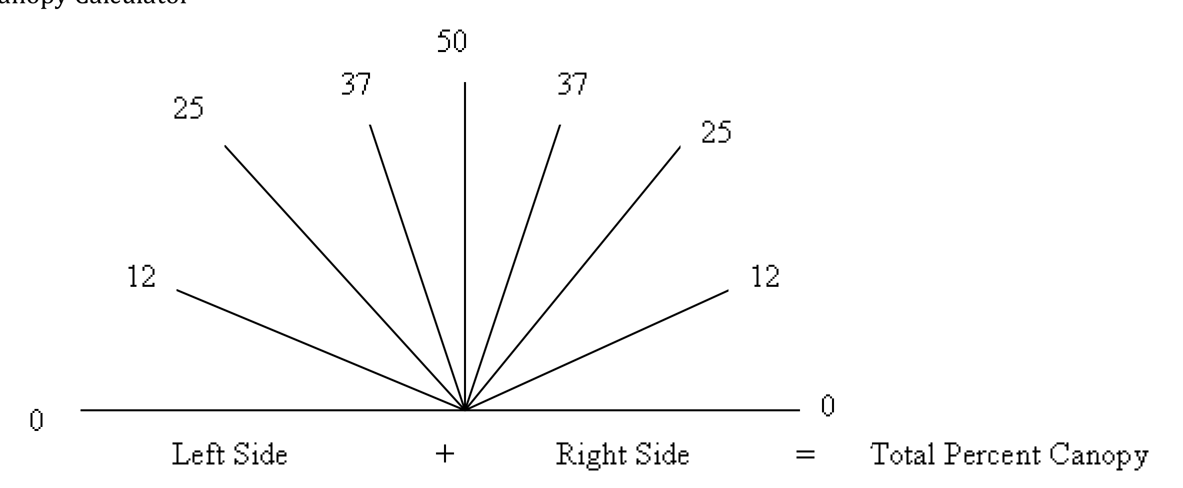
3. Averting (closing) one's eyes, a pebble is selected by touching the bottom with ones index finger. The randomly selected pebble is then placed in a particle size category. Size categories were initially based on the Wentworth's size classes, which were then lumped into larger biologically based size classes used by the VTDEC to describe substrate composition. The VTDEC size categories are: Sand **<2mm** (.08"), Gravel **2-16mm**(.08-2.5"), Course Gravel **16-64mm** (.63-2.5"), Cobble **64-256mm** (2.5-10.1"), Boulder **>256mm** (>10.1").

4. Size categories are determined by using a gravelometer, essentially a metal plate with squares of the above size classes cut out. The particle must be placed thru the smallest cut out so that the intermediate axis is perpendicular to the sides (not diagonally across) of the cut out. The smallest size category, which the pebble falls through is called out to a recorder, who keeps track of the tally until the minimum of 100 particles is reached. If this occurs in the middle of a transect, it is completed.

Percent Canopy Measurement Guidance

Stand in center of stream/river, extend both of your arms straight out creating a 180-degree angle. Observing the overhead canopy cover, start to lift your arms up from the straight out position slowly towards your head. Stop when each arm is in alignment with the overhead canopy.
Then estimate the angle of your left and right arm using the figure below for guidance. Combine the percent canopy values from the left and right side to obtain the total percent canopy.

Percent Canopy Calculator



Laboratory Method: Identifying, Preserving, & Counting Macroinverts

1) Pour contents of one individual sample (usually one Whirl pak ®) into a bucket. Add water and gently pour organic matter into a #30 sieve to remove all excess ethanol. Continue to add water and swirl sample until organic matter has been removed and sand remains in bucket. This will be processed later.

2) Spread organic matter evenly over a tray that is divided into 12-squares. Add a small amount of water to the tray to allow the sample to be evenly spread, but not so much as to cause the macroinvertebrates to float freely around the tray.

3) Randomly choose a number between 1 and 12, which will correspond to a square on your tray (use 12-sided dice or this excel formula: =int(rand()*12)+1). Use dominoes to separate this square and the next 3 consecutive squares from the rest of the sample. This will represent one quarter of your sample. Alternatively, generate a series of random numbers and work through the list until you've reached 4 squares or 75 insects. Pick all organisms from the selected sections with the aid of a 2x magnifier while keeping a tally. Completely pick each of the 4 squares (i.e., do not leave any insects remaining). For bugs which are not intact, only tally the heads not other body parts which are found.

4) After the 4 squares have been completely picked either take a break and check the area again later for bugs which have been missed, or have someone else check your sample. If after this time the minimum number of 75 organisms from the sample has not been reached, pick additional grids on the tray to reach that number. **Record the total number of grids (squares) that were picked** so that sample density or relative abundance can be calculated.

5) Sort animals into major groups, and preserve in 75% ETOH with 1% glycerin.

6) Using the keys provided, identify each individual to genus/species (depends on reference collection) except for the Chironomidae and Oligochaeta which will not be identified beyond the family and subclass level, respectively.

7) Store identified insects in a plastic vial with label indicating information on the site name, replicate sample number, date, identification (**in pencil or laser printed**).

8) Keep a record of all identification data on your Benthic Macroinvertebrate data sheet and enter the data online.

Macroinvertebrate Field Labels

*Please use labels provided by the Streams Staff

*Labels should be complete **IN PENCIL** and places **INSIDE** the whirlpak. Labels filled out with markers will be erased by the ethanol!

Site Code
Replicate Number
Collectors
School Name
Sample Date
Major Drainage
Stream Name
Town
Nearest Street
Sample ID Number
(yy/mm/dd/Rep #)

Benthic Macroinvertebrate Habitat Data Sheet

2011-2012

Stream Name:	Site Code:
Latitude/Longitude:	Date/Time:
Site Description:	Investigators:

Sample Collection	Time spent collecting each replicate sample (should be 30 seconds): 1) 2) 3) 4) Comments:
Field Measurements	Air Temp: ___°C Water temp: ___°C pH: _____ Velocity (m/s) (at mid-point): _____ Bank full width (m): ___ Wetted width (m): _______ Depths where samples collected(m): 1) 2) 3) 4) Canopy cover: 100 90 80 70 60 50 40 30 20 10 0 %

PEBBLE COUNT

Particle	**Millimeters**	**Transect 1 (100 pebbles)**	**Total #**	**Item %**
Clay/Silt/Sand	< 0.004-2.0			
Gravel	2.0-16			
Coarse gravel	16-64			
Cobble	64-256			
Boulder	> 256			
Bedrock				
		Totals:		

Comments:

Macroinvertebrate Data Sheet
2011-2012

Sample ID Number (yy/mm/dd/Rep #): ____________________
Site Code: ____________________

Watershed: __________________ Town: ___________ Location: ________________
Picked by: ____________________ Date Picked: _____________

Number Bugs Found: __________ Number Squares Picked: ________________

Sorted By: ________________ Date Sorted: ______________
IDed by: __________________ Date IDed: ________________

Date	Initials	Class	Order	Family	Notes	#

Section 4: Data

Contents

Uploading Field Data....2

Uploading Macroinvertebrate Data....3

Viewing and Downloading Data....4

Data Analysis Overview....5

Macroinvertebrate Data - Community Analysis....6

Uploading Field Data

Undergraduate and high school teams are responsible for uploading all of their data into the online database with the exception of the lab results for *E. coli*, total suspended solids, and total phosphorus. The lab data will be added to the online database by Streams Project staff once it has been analyzed in the lab. The data that you are expected to upload correspond to the following field sheets:

- Habitat assessment
- Site assessment
- Benthic macroinvertebrate field data
- Water quality assessment data

Additionally, you are expected to enter the following:

- Taxonomic information on the macroinvertebrates collected at your stream sites. (Instructions in next section)

To keep on top of entering the data, ***enter your data within a month of collecting the data*** in the field. By January, Streams Project staff will begin to check in with teams who have not been entering data.

To enter data into the online database, contact streams@uvm.edu

Once you are at the web page:

1. Select your stream site code under "Stream/Site Name." You'll notice four boxes appear with the following titles: Site Assessment Data, Habitat Assessment Data, Water Quality Assessment Data, and Macroinvertebrate data. These correspond to the four field sheets mentioned above. The dates in each box represent the collection date of previous entries for this site.

2. **To add** a new entry, select the "New" button for the data you would like to enter. Fill out the electronic sheet which corresponds to your field sheet for the respective data. Click "submit" when you have finished.

3. **To edit** an already submitted field sheet, select the date of the entry in the box and then select the "Edit" button. Make the necessary changes and then click "submit."

4. **To just view** a field sheet that has already been submitted, select "View." Click the "Site Summary" page to go back to the initial view.

Uploading the data to the online database is an important step in growing our database and making it available Streams Project participants and other interested parties. The recommendation for monthly uploading will help ensure we keep the flow of data coming!

If you have any questions about uploading data, please contact streams@uvm.edu

Uploading Macroinvertebrate Data

Additionally, you are expected to enter the following taxonomic information on the macroinvertebrates collected at your stream sites

To enter your macroinvertebrate taxonomic data, please use the following web page:

To enter data into the online database, contact streams@uvm.edu

Once you are at the web page:

1. **Select your** stream site code under "Stream/Site Name", select your school or organization under "School/Organization", and select "Replicate Number".
2. **Fill in** the information "Number of Squares Picked", "Sample ID Number", "Sorted By", "Date Sorted", "Time & Date Collected", "IDed by", "Number of Bugs", and "Date IDed" from the macroinvertebrate label and Macroinvertebrate Data Sheet sheets.
3. **To add** insect macroinvertebrate counts for your replicates, click the "Insect." Then select the proper "Order" and "Family", and finally enter the number of insects under "Count." To add a new insect species click the down arrow to the left of "Count." PLEASE only enter macroinvertebrate information manually for which there is no option under the drop down menu.
4. **To add** non-insect macroinvertebrate counts for your replicates, leave "Insect" box unchecked. Then select the proper "Phylum", "Class", "Order" and "Family", and finally enter the number of insects under "Count." To add a new macroinvertebrate species click the down arrow to the left of "Count." PLEASE only enter macroinvertebrate information manually for which there is no option under the drop down menu.
5. **Check and review** data and information entered into the Streams Macroinvertebrate Input Form. Click "submit" when you have finished.

Uploading the data to the online database is an important step in growing our database and making it available Streams Project participants and other interested parties. The recommendation for monthly uploading will help ensure we keep the flow of data coming!

If you have any questions about uploading data, please contact streams@uvm.edu

Viewing and Downloading Data

To view or download data in the Streams Project's database, go to the following location on the Streams Project's website:

http://www.uvm.edu/~streams/?Content=pages/download_data.inc

Once you are at the web page:

1. Select the stream sites for which you'd like data. If you'd like a handful, hold down the "Ctrl" button in between selections. If you'd like data for all the streams sites, select the first stream site, hold down the "Shift" button, and the select the last stream site in the list.
2. Select the report that represents the type of data you are interested in under "Available Reports."
3. Select the date range for which you'd like data.
4. Once you've made these selections click the "Generate Report" button.
5. You can view the data available for these criteria on the webpage that appears. If you click on the heading of a data field in the table, a little box will pop up describing the data contained in that field.
6. To download the data seen here, click the "Export to Excel File" text above the table and save the file on your local computer.

An explanation of the data in the database, and a description of how to download data from this web page can also be found in **Module 3: Refining and Retrieving Data** of the Data Analysis Tutorial. The link to this module can be found here:

http://www.uvm.edu/~streams/?Content=pages/data_analysis_tutorials.inc

The next section on data analysis explains the use of this tutorial further.

Data Analysis Overview

You should begin thinking about your preparing your poster or presentation for the Streams Project Symposium in April as soon as possible. The basis of your poster or presentation will be an analysis of the data you have gathered for your streams sites over the past year AND the data that are already in the online database. You may also chose to look beyond just your stream sites at the whole dataset, or a subset of data found in the Streams Project's database. Remember, this includes the site assessment data, habitat assessment data, the bi-monthly water quality data and your macroinvertebrate field data and samples.

The Streams Project has created a **data analysis tutorial** to help guide you through the process of exploring and asking more in-depth analysis questions about your dataset. This should be your primary guide for beginning your data analysis, but the Streams Project staff members are always available to help you along the way. The tutorial can be found on the Streams Project website here:

http://www.uvm.edu/~streams/?Content=pages/data_analysis_tutorials.inc

The first link on the page that says "Complete Tutorial Series - All Modules" will open a pdf with all of the modules compiled into one document. The subsequent links are for accessing modules individually. The following is a list of the individual modules and what they cover:

- Module 1: What is science?
- Module 2: Understanding Streams Project Data
- Module 3: Refining and Retrieving Data
- Module 4: Data Exploration
- Module 5: Statistical Analysis
- Module 6: Summarizing Results and Drawing Conclusions

In this tutorial, statistical analysis is demonstrated using Microsoft Excel. Within each module, look for the "WATCH VIDEO" icon that looks like this:

These videos help you visualize a number of procedures outlined in the tutorial. ****NOTE:** To be able to watch the videos, download the QuickTime Player, if it is not already on your computer: http://www.apple.com/quicktime/download/

Macroinvertebrate Data - Community Analysis

Analysis of the community of macroinvertebrates found in your streams is not specifically addressed in the data analysis tutorial. The calculation of metrics that will help you interpret your macroinvertebrate taxonomic data is a necessary first step before further analysis as described in the Data Analysis Tutorials. For the Streams Project, we're investigating differences among streams of varying surrounding land use, so metrics such as taxa richness (taxa = species, or lowest level of identification for your samples, i.e., family, genus), composition, and functional feeding groups of your samples are relevant to consider. (A classification of the taxa by functional feeding groups can be found in Appendix A of the University of Minnesota "Guide to the Aquatic Invertebrates of the Upper Midwest", which is included in your packet).

The Calculation of Metrics document to follow here is a resource on calculating metrics that describe the macroinvertebrate communities found in the streams you are examining through your analysis. The document details the meaning and application of eight commonly used metrics to describe macroinvertebrate communities.

You do not have to use all of these metrics, so chose one of particular interest or relevance to your study question. We are happy to help along the way, but please use this resource to get you started!

Calculation of Metrics

1. Density- Is the relative abundance of animals in a sample.

Calculation: Number of animals in subsample / proportion of sample processed.

Example : 300 animals picked / 0.25 (or one quarter of sample picked) = 1200 animals/sample

2. Richness- Species richness is the number of species in a sample unit.

Calculation: Richness is the total number of distinct taxa identified in a sample. Note immature larva identified to family or genus are not considered a distinct new taxa if a genus or species identification is determined within its group.

Example :

Taxon	# orgs Rep 1	# orgs Rep 2
Ephemerellidae Ephemerella sp	2	0
Ephemerellidae Ephemerella dorothea	3	4
Ephemerellidae Ephemerella invaria	0	2
Richness =	1	2
Mean Richness =	1.5	

3. EPT Index- The EPT index is a subset of the above richness measure. It is the number of species in the sample in the generally more environmentally sensitive orders Ephemeroptera, Plecoptera, and Trichoptera.

Calculation: The number of distinct taxa identified in a sample from the insect orders Ephemeroptera, Plecoptera, Trichoptera. Note same rules apply as above for richness in determining number of distinct taxa.

4. EPT/EPT & Chironomidae - Is a measure of the ratio of the abundance of the intolerant EPT orders to the generally tolerant Diptera family Chironomidae.

Calculation: The number (abundance) of animals from the orders Ephemeroptera, Trichoptera and Plecoptera, divided by the above plus the number of Chironomidae.

5. % Oligochaeta - Is a measure of the percent of the macroinvertebrate community made up of the Order Oligochaeta.

Calculation: The number (abundance) of Oligochaeta divided by the total number of animals in sample.

6. Percent Model Affinity of Orders - (PMA-O) Is a measure of order level similarity to a model based on the reference streams Novak and Bode (1992).

Calculation: Determine the percent composition for each major group - Coleoptera, Diptera, Ephemeroptera, Plecoptera, Trichoptera, Oligochaeta, Other. Compare to the "Model" for the appropriate stream community (see below), then add up the lower of the two values for each of the groups (assessment site vs Model), this is the PMA-O for the assessment site.

PMA-O = 3 min (X_a or X_r)

Where: X_a = the percent composition of order X from the assessment site;

X_r = the percent composition of order X from the appropriate reference condition;

Example:

Percent Composition Major Grps	Assessment Site % Comp	Model for MMC (Medium Mt)
Coleoptera	20	**6**
Diptera	55	**18**
Ephemeroptera	**10**	34
Plecoptera	**2**	8
Trichoptera	**3**	33
Oligocheata	10	**0.5**
Other	**0**	0.5
PMA-Orders =	39.5 rounded = 40.0	

7. Hilsenhoff Biotic Index- BI (0-10) - Is a measure of the macroinvertebrate assemblage tolerance toward organic (nutrient) enrichment Hilsenhoff (1987). In many ways this index is both an indicator taxa metric and functional group metric, since those taxa which become more dominant in moderately enriched streams are those which are taking advantage of shifts in the available food base in the stream.

Calculation : Multiply the number of individuals of a taxon by its assigned tolerance value, see VTDEC BI values, modified from Hilsenhoff 1987, and Bode 1996. Total all these products, and divide by the total number of individuals <u>of each taxon assigned a tolerance value</u>. This is the Bio Index value.

$$HBI = 3 \ \frac{n_i \, a_i}{N}$$

Where:"n" is the number of individuals of the "i"th taxon;

"a" is the index value of that taxon;

N is the total number of individuals in the sample assigned a Bio Index Value

Example :

Taxon	Count	BI Tolerance Value	Subtotal Ct × BI
Ephemerllidae imm	(10)	NA	NA
Ephemerella sp	**10**	4	**40**
Ephemerella needhami	**10**	1	**10**
Plecoptera Leuctridae imm	**20**	0	**0**
Diptera Cricotopus bisinctus	**5**	6	**30**
Trichoptera Symphitopsyche alhedra	**10**	3	**30**
Trichoptera Symphitopsyche sp	**5**	5	**25**
Totals	***60***		***145***
Site Bio Index Value	145 ÷ 60 = ***<u>2.42</u>***		

8. Pinkham-Pearson Coefficient of Similarity - Functional Groups - (PPCS-F) - Is a measure of functional feeding group similarity to a model based on the reference streams. It is similar in concept to the **PMA-O** in that a site is compared to a model of the composition of the functional feeding groups as opposed to order level taxonomic changes. Also the Pinkham Pearson Coefficient of Similarity (Pinkham1976) was used as the similarity index.

Calculation: At the assessment site determine the percent composition of the six major functional groups (Collector Gatherer, Collector Filterer, Predator, Shredder-Detritus, Shredder-Herbivore, Scraper) as assigned by VTDEC after Merrit and Cummins 1996, Bode 1996. For each functional group determine the product (min/max) between the assessment site vs the Model for the stream community sampled. Add these products and divide by six (# of functional grps). This is the PPCS-F.

$$\text{PPCS-F} = 1/k \sum_{I=1}^{K} \text{minimum}(xia, xib)/\text{maximum}(xia, xib)$$

Where: k = the number of comparisons between stations (6)

xi = the number of individuals in functional group I

a, b = site a, site b

Example :

Functional Group	Assessment Site % Comp	"Model" for MMC	Product (min/max)
Collector .Gatherer	68	32	0.47
Collector Filterer	10	30	0.33
Predator	2	13	0.15
Shredder - Detritus	0	4	0.00
Shredder - Herbaceous	16	1	0.06
Scraper	2	13	0.15
PPCS-F =			**0.19**

Section 6: Field Safety

Contents

First Aid Kit....2

Disinfecting Waders....3

Didmyo Fact Sheet....4

Field Precautions....5

Poison Parsnip....5

Poison Ivy....6

Ticks & Lyme Disease....7

First Aid Kit

When working in the field, it is important to be prepared for emergencies. Although you will not be traveling far from your car when you visit your field sites for the VT Streams Project, accidents may still happen. Therefore, a well-stocked first aid kit is an important thing to have. Carry a first aid kit with you to your site or keep one in the car. You may purchase a pre-made kit at the store, or you may make your own using the recommended list of items below as a reference. Whichever you chose, it is important to include any personal items such as medications and emergency phone numbers. Check the kit regularly and replace any used or out-of-date items.

- Adhesive bandages (assorted sizes)
- Antibiotic ointment
- Antiseptic wipes
- Instant cold compress
- Hydrocortisone ointment
- Scissors
- Sterile gauze pads (assorted sizes)
- Butterfly bandages
- Tweezers
- Prescription medications (asthma inhalers, Epipen)
- Emergency phone numbers
- Charged cell phone

Disinfecting Waders

We have supplied your team with concentrated Quaternary Ammonium Disinfectant (Quat solution) to kill and prevent the spread of nuisance biological agents such as Didymo. This procedure is adapted from the Vermont Agency of Natural Resources method for equipment disinfection.

****ATTENTION: Quat is a highly basic solution. Protective gloves MUST be worn when handling the concentrated solution. Once diluted with water, it is safe to handle****

To prepare a 2.5% solution:

- Add 25mL of concentrated Quat to a spray bottle. Dilute to 1L. (For 500mL of solution, add 12.5mL of concentrated Quat and dilute with water to 500mL.) **Quat solutions should be replaced every 2 – 3 days to remain effective, so prepare only as much as is necessary for a site visit.**

- Fill the second spray bottle with water.

- When exiting the stream following sampling, spray waders and other equipment thoroughly with the 2.5% Quat solution. Let sit for ~2 minutes. Spray with the water to rinse.

Didmyo Fact Sheet

Didymosphenia geminate, commonly known as "Rock Snot" or "Didymo," is an aggressive freshwater alga that has undergone a recent large expansion in range. It has the potential to form nuisance blooms during which it can form mats several inches thick by attaching itself to streambeds by stalks that form a thick brown mat on rocks, plants, and other aquatic surfaces. The thick growth reduces the quantity and quality of aquatic habitat.

Didymo was detected in rivers of Vermont, New York, and New Hampshire during the summers of 2006 and 2007. Because the factors that cause Didymo to undergo rapid growth are unknown and there is no known method of eradication, it is important to prevent the spread of these algae to uninhabited streams. Therefore, we disinfect all waders and equipment when traveling between streams. In order to prevent the spread of didymo to other regions waders should not be transported and used in different regions or countries.

Follow the link for a detailed description of Didymo by the Vermont Department of Environment Conservation Water Quality Division:

http://www.anr.state.vt.us/dec//waterq/lakes/htm/ans/lp_didymo.htm#how_can_I_disinfect

Field Precautions

Poison Parsnip

- **Location**: Predominately found on the sides of highways and fields throughout Vermont.

- **Appearance**: The plants typically grow 3-6 feet tall and resemble Queen Anne's Lace, but the flowers are yellow instead of white.

- **Danger**:
    - The plant contains a high concentration of furocoumarin chemicals
    - The plant's juices may be transferred to your skin if you brush against the flower tops or broken leaves or stems
    - When the juices on the skin are exposed to ultraviolet light on both sunny and cloudy days the furocoumarin chemicals bind with nuclear DNA and cell membranes.
    - **This process destroys cells and skin tissue, causing severe burns in which the skin to reddens and blisters**

- **Protecting Yourself:**
    - Avoid exposure to the plant by choosing stream sites or access areas free from poison parsnip
    - If unavoidable, wear long sleeve shirts, pants (or your waders!), and gloves to prevent direct contact with your skin
    - Rinse and wash all clothing items and skin surfaces immediately following possible exposure. Keep exposed skin out of sunlight.

Poison Ivy

Poison ivy in spring.

Image © Jonathan Sachs 2002

Avoid poison ivy

Preventing contact with poison ivy

- Do not touch or handle any part of the plant
- Remove and wash shoes or clothing that has contacted poison ivy. Wash your hands immediately with soap and water

Preventative treatment Modified From http://poisoncontrol.uchc.edu

- If you have touched poison ivy, avoid spreading the oils to other body parts and wash the affected skin with soap and water within 15 minutes
- Use a nail brush to clean under finger nails
- Swab with rubbing alcohol after washing

Poison ivy in summer.

www.kentuckycrosswords.com

Myths Vs Facts: Fact #1: this fact list is modified from www.zanfel.com

Myth: Scratching poison ivy blisters will spread the rash.
Fact: Fluids from blisters will not spread the rash. Before blisters form, the rash can only be spread by unbound urushiol. Scratching of blisters can cause bacterial infection.

Myth: Poison ivy rash is "contagious."
Fact: The rash is a reaction to urushiol. The rash cannot pass from person to person after the urushiol binds to skin.

Myth: After the first time, I can't get poison ivy again.
Fact: Not everyone reacts to poison ivy upon first or subsequent exposures, people generally become more sensitized with each contact and may react more severely to subsequent exposures.

Myth: Once allergic, always allergic to poison ivy.
Fact: A person's sensitivity changes over time, even from season to season. People who were sensitive to poison ivy as children may not be allergic as adults.

Myth: Dead poison ivy plants are no longer toxic.
Fact: Urushiol remains active for up to five years. Never handle dead plants that look like poison ivy without proper protection.

Myth: Burning is the best way to dispose of poison ivy.
Fact: The toxic oils from poison ivy spread in the smoke and can cause full-body rash and more serious health problems if inhaled.

Zanfel Laboratories provides poison ivy treatment brochures for free to BSA troops. Call 1800 401 4002

If a rash develops From http://poisoncontrol.uchc.edu

- Apply calamine lotion, cool compresses, or over the counter corticosteroid creams to lessen itching. Oatmeal baths can also help. Avoid scratching and cover open blisters to avoid infection. If face or genitals are involved, see a doctor for evaluation. If symptoms are persistent after these treatments see a doctor.

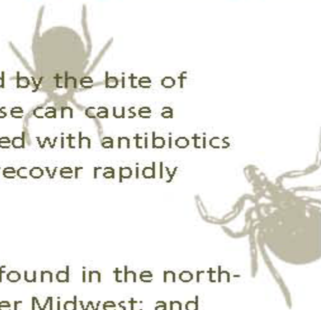
Leaf size varies greatly.

Ticks & Lyme Disease

T I C K S & L Y M E D I S E A S E

What Is Lyme Disease?

Lyme disease is a bacterial infection caused by the bite of an infected deer tick. Untreated, the disease can cause a number of health problems. Patients treated with antibiotics in the early stage of the infection usually recover rapidly and completely.



Where Is Lyme Disease Found?

In the United States, infected ticks can be found in the north-east, including New York State; in the upper Midwest; and along the northwest coast.

What Are the Symptoms of Lyme Disease?

The early symptoms of Lyme disease may be mild and easily missed. If you find a tick attached to your skin, remove the tick with tweezers and watch for the symptoms of Lyme disease. In 60-80% of cases the first symptom is a rash, known as *erythema migrans,* that:

- Occurs at or near the site of the tick bite.
- Is a "bulls-eye" circular patch or solid red patch that grows larger.
- Appears between three days and one month after the tick bite.
- Has a diameter of two to six inches.
- Lasts for about three to five weeks.
- May or may not be warm to the touch.
- Is usually not painful or itchy.
- Sometimes multiple rashes appear.

1

How Can I Safely Remove a Tick?

If you DO find a tick attached to your skin, do not panic. Not all ticks are infected, and your risk of Lyme disease is greatly reduced if the tick is removed within the first 36 hours.

To remove a tick:

- Use a pair of pointed tweezers to grasp the tick by the head or mouth parts right where they enter the skin. DO NOT grasp the tick by the body.
- Pull firmly and steadily outward. DO NOT jerk or twist the tick.
- Place the tick in a small container of rubbing alcohol to kill it.
- Clean the bite wound with rubbing alcohol or hydrogen peroxide.
- Monitor the site of the bite for the next 30 days, for the appearance of a rash. If you develop a rash or flu-like symptoms, contact your health care provider immediately.

What Else Can Be Done?

- Keep lawns mowed and edges trimmed.
- Clear brush, leaf litter and tall grass around the house, and at the edges of gardens and stone walls.
- Stack woodpiles neatly away from the house and preferably off the ground.
- Clear all leaf litter (including the remains of perennials) out of the garden in the fall.
- Keep the ground under bird feeders clean so as not to attract small animals.
- Locate children's swing sets and other play equipment in sunny, dry areas of the yard, away from the woods.

For more information on Lyme disease, contact your local health department or refer to the NYS Department of Health web site at ***www.health.state.ny.us***

6

- Do NOT apply repellents directly to children. Apply to your own hands and then put it on the child.
- When applying repellents, avoid the child's face and hands.
- Do not apply repellents on skin damaged by sunburn, cuts, bruises or other conditions, such as psoriasis.
- Avoid prolonged and excessive use of DEET.
- Do NOT apply repellents in enclosed areas.
- Do NOT apply directly on your face.
- Do NOT apply near eyes, nose or mouth.
- Wash treated skin and clothing after returning indoors.
- If you believe you or a child is having an adverse reaction to a repellent containing DEET, wash the treated area immediately and contact your local health care provider or local poison control center.

Also consider these important facts:

- If you tuck pants into socks and shirts into pants, be aware that ticks will climb upward to hidden areas of the head and neck, so spot-check clothes frequently.
- Clothes can be sprayed with DEET or treated with permethrin. Follow label instructions carefully.
- Upon returning home, clothes can be put in a high temperature dryer for 20 minutes to kill any unseen ticks. A shower and shampoo may help to dislodge crawling ticks, but this is not always effective.
- Any contact with vegetation, even playing in the yard, can result in exposure to ticks. Frequent tick checks should be followed by a whole-body examination and tick removal each night. This is the single most effective method for prevention of Lyme disease.

Ticks will attach themselves anywhere including the thighs, groin, trunk, armpits and behind the ears. If you are infected, the rash may be found in one of these areas.

Around the time the rash appears, other symptoms, such as joint pain, chills, fever and fatigue can occur, but they may seem too mild to require medical attention. As Lyme disease progresses, severe fatigue, a stiff aching neck, and tingling or numbness in the arms and legs, or facial paralysis can occur.

The most severe symptoms of Lyme disease may not appear until weeks, months or years after the tick bite. These can include severe headaches, painful arthritis, swelling of the joints, and heart and central nervous system problems.

How Is Lyme Disease Diagnosed?

If you think you have Lyme disease, you should see your health care provider immediately. Early diagnosis of Lyme disease should be made on the basis of symptoms and history of possible exposure to ticks. Blood tests may give false negative results if performed in the first month after the tick bite.

How Is Lyme Disease Treated?

Early treatment of Lyme disease involves antibiotics and almost always results in a full cure. However, the chances of a complete cure decrease if treatment is delayed.

In a small number of cases, Lyme disease can become a chronic condition. However, some patients have reported slow improvement and even an end to symptoms, months or even years after treatment.

How Can I Protect Against Ticks and Prevent Lyme Disease?

Deer ticks live in shady, moist areas at ground level. They will cling to tall grass, brush and shrubs, usually no more than 18-24 inches off the ground. They also live in lawns and gardens, especially at the edges of woods and around old stone walls.

Deer ticks cannot jump or fly, and do not drop onto passing people or animals. They get on humans and animals only by direct contact. Once a tick gets on the skin, it generally climbs upward until it reaches a protected area.

In tick-infested areas, your best protection is to avoid contact with soil, leaf litter and vegetation. However, if you garden, hike, camp, hunt, work, or otherwise spend time in the outdoors, you can still protect yourself:

- **Wear light-colored clothing** with a tight weave to spot ticks easily.
- **Wear enclosed shoes, long pants and a long-sleeved shirt.** Tuck pant legs into socks or boots and shirt into pants.
- **Check clothes and any exposed skin frequently** for ticks while outdoors.
- **Consider using insect repellent.**
- **Stay on cleared, well-traveled trails. Avoid contacting vegetation.**
- **Avoid sitting directly on the ground or on stone walls.**
- **Keep long hair tied back,** especially when gardening.
- **Do a final, full-body tick check at the end of the day** (also check children and pets), and remove ticks promptly.

What Do Ticks Look Like?

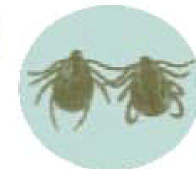
Two common types of ticks are dog ticks and deer ticks. Deer ticks can carry Lyme disease. Dog ticks can carry Rocky Mountain spotted fever but have not been known to carry Lyme disease.

Deer Ticks Actual Size

Enlarged View Female Deer Tick

Female deer ticks have four pairs of legs and are red and black in color, while the male is all black. Young deer ticks - nymphs, are brown, the size of poppy seeds and very difficult to spot. An adult deer tick is only about the size of a sesame seed – still very small.

Adult Dog Tick Actual Size


Enlarged View, Male and Female Dog Ticks

Dog ticks are the most common type of tick, and, while feeding, can be as large as a small pea. They have four pairs of legs, are reddish-brown and are easier to spot. Dog ticks turn gray while feeding. Ticks can be found throughout the year, but they are most active during the spring, early summer and fall, when it is warm and moist.

What About Insect Repellent?

Two active ingredients found in repellents are DEET (the label may say N, N-diethyl-m-toluamide) and permethrin. Permethrin is only used on clothes. DEET repellents or products come in many different concentrations, with percentages as low as five percent or as high as 100 percent. In general, the higher the concentration the higher the protection, but the risk of negative health effects goes up too. Use the lowest concentration that you think will provide the protection you need. The New York State Health Department recommends taking these precautions when using repellents that contain these active ingredients:

- Store out of the reach of children and read all instructions on the label before applying.
- Do NOT allow children to apply repellents themselves.