UNIT 1. Sampling Local Biodiversity

Overview: Collecting local samples of interest is the first step in generating a DNA barcode. Sample collection can be done just about anywhere but what matters most is documenting information about how and where the samples were collected. This curriculum introduces students to sampling invertebrates at a local park along with the materials and methods used for sampling. For this unit we have included data sheets, information on calculating biodiversity using the Simpson's Diversity Index, and an outline and rubric for writing up a final scientific paper.

1. *Developing a method for sampling local biodiversity:* Before collecting samples, a general question should be generated as a class. Overarching questions about local biodiversity can include anything related to the collection of biological material from a variety of locations including but not limited to parks, markets (and other stores), and zoos. For this curriculum, we asked students to try to determine the biodiversity of insects at a local park in New York City. Students researched about the different types of ecosystems in the park, randomly generated plots using Google Maps, determined the best collection method for their ecosystem, and generated data sheets.

Examples of Activities for Developing a Sampling Method:

A. *Introduction to Local Ecosystems:* Students read about local ecosystems in New York City, the characteristics of that ecosystem, the types of animals and plants that exist there, and local examples. Students create short presentations for each ecosystem and share with the class.

B. *Generating Random Samples:* Students use Google Maps to find where their ecosystem of interest is located within the park. Students outline the area on graph paper, number the squares, and use a random number generator to select three locations within the park to sample. Students get the GPS coordinates for each location and use those during the field collection days.

C. *Developing Collection Methods:* Students are given entomology books and collection equipment to become familiar with. Each ecosystem uses their knowledge of their ecosystem and the collection resources to develop two collection methods for insects. Students design their own traps and baits in class prior to field collection days.

2. *Fieldwork and Sample Collection:* After determining the method for sampling local biodiversity, fieldwork is conducted to collect and preserve samples. Depending on the question being asked collection and fieldwork will vary. For this curriculum we brought students to a local park on three

weekend days for about six hours of fieldwork. The first field day allowed students to become oriented to the park, make observations of the insects in their ecosystem, and locate and flag their sample plots. The second and third trips were for collection of insects.

A. Insect Collection Materials: To collect insects from a local park there are some basic materials that must be used. Additionally, any materials that students need for their own traps should be included.
    - GPS unit
    - Camera
    - Insect nets
    - Collection Jars and envelopes
    - Kill jars
    - Acetone for killing
    - Tweezers
    - Hand lens
    - Labeling marker
    - Field Guides
    - Materials for traps

B. Data Sheets: Generating good data sheets will make it easier for students to organize their samples while working in the field. We are providing two sample data sheets. The first we used at a local park collecting insects (1.2a) and the second we used for students in Belize collecting fish fillets (1.2b).
C. Killing and Labeling Samples: Killing insects in the field requires the use of acetone in a kill jar. Add a few drops of acetone to the kill jar, close the lid to let the fumes build up, insert the insect. Once the insect has been killed, move into a collection jar. In the field it is important to make sure that students keep track of samples. All samples must be labeled with the following information:
    - Insect ID Number
    - Location
    - Date
    - Collector Name
    - Collection Method

3. *Post-trip Tasks:* After returning to the classroom with samples, each sample must be processed. For this curriculum processing includes recording observations of the insect, identification to the lowest level, pinning of insects, photographing each insect from the top and side view, removing a leg for DNA barcoding, and documenting all of the insects on our class website.

SAMPLE POST-TRIP CHECKLIST:

Task	**Completed**
1. Upload trip photos to Picasa and share	
2. Transfer data table(s) into notebook	
3. Observe insects and try to identify to the lowest level - for each specimen keep records in your notebooks of the characteristics/drawings/names	
4. Pin insects	
5. Photograph each insect after it is pinned- top shot with ruler, side shot	
6. Upload all pictures to Picasa account – edit pictures (crop, brighten, enhance)	
7. Remove leg (back right) and place into ethanol tube with DNA barcode label=specimen ID number	

8. Type up and print labels for each specimen	
9. Upload all photos and collection information to class website	

SAMPLE STUDENT DNA BARCODING DATA:

Specimen ID#	Country	City	Latitude	Longitude	Date Collected	Collector Name	Collection Method	Insect Order	DNA Barcode ID
IHPG541	USA	New York	40.877467 N	-73.926412 W	29-Sep-2012	M. E. Bellino	Sweep Net	Hymenoptera	41
IHPG542	USA	New York	40.877467 N	-73.926412 W	29-Sep-2012	B. A. Font	Bare Hands	Diptera	42
IHPG543	USA	New York	40.877467 N	-73.926412 W	13-Oct-2012	E. McKan	Sweep Net	Diptera	43
IHPG544	USA	New York	40.877467 N	-73.926412 W	13-Oct-2012	J. Ramtel	Sweep Net	Lepidoptera	44
IHPG545	USA	New York	40.877467 N	-73.926412 W	29-Sep-2012	M. Poppy	Tweezers	Hymenoptera	45
IHPG546	USA	New York	40.877467 N	-73.926412 W	29-Sep-2012	M. E. Bellino	Under rock	Dermaptera	46
IHPG547	USA	New York	40.877467 N	-73.926412 W	29-Sep-2012	E. McKan	Tweezers	Dermaptera	47
IHPG548	USA	New York	40.877467 N	-73.926412 W	13-Oct-2012	J. Ramtel	In sand (Kill Jar)	Hymenoptera	48
IHPG549	USA	New York	40.877467 N	-73.926412 W	13-Oct-2012	M. Poppy	Sweep Net	Hymenoptera	49
IHPG550	USA	New York	40.877467 N	-73.926412 W	13-Oct-2012	M. Poppy	Sweep Net	Diptera	50

SAMPLE INSECT LABELS:

TOP LABEL:	MIDDLE LABEL:	BOTTOM LABEL:
Location: COUNTRY, State, City Latitude, Longitude Date (Day, Month, Year) Collector Name (Last Name, First and middle initials)	Insect Identification	DNA Barcode ID #

Example:

TOP LABEL:	MIDDLE LABEL:	BOTTOM LABEL:
BELIZE, Dangriga 16°58.468'N, 088°13.327'W 24 July, 2012 Rancharan S.	Family Salticidae	DNA Barcode ID #06

4. *Calculating Biodiversity:* Even if students cannot identify all the samples, it is possible to differentiate between species based on morphological characteristics. Students can use these differences to calculate the biodiversity of insects using the Simpson's Diversity Index (1.4a).

5. *Writing up a final report:* Writing scientifically takes practice. All of the student research is written up into a final paper in the style of a journal article (1.5a, 1.5b). Sample articles are given to students to help with language, style, and formatting.

1.2a. INWOOD HILL PARK: DATA SHEET

CHALLENGE: How can we determine the biodiversity of invertebrates at Inwood Hill Park?
This is a sample of what should be in each of your lab notebooks. You can fill this in as a group during your collection and copy into each of your lab notebooks back in the classroom.
Remember to take pictures.

Date _____

Weather _____

Group # _____

Group members: _____

Ecosystem: _____

Plot 1: Latitude: _____ Longitude _____

Plot Description: _____

Insect ID #	Collected by	Collection Method	Additional Notes (include photo info)

Plot 2: Latitude: _____ Longitude _____

Plot Description: _____

Insect ID #	Collected by	Collection Method	Additional Notes (include photo info)

Plot 3: Latitude: _____ Longitude _____

Plot Description: _____

_____

_____

_____

_____

Insect ID #	Collected by	Collection Method	Additional Notes (include photo info)

1.2b. DATA SHEET FOR SAMPLE COLLECTION

Type of Study:

- Investigative (Unknown sample/species) or Reference (Species Known)

Identification Code:

- Year-InstitutionCode-Initials-Number (example, 13-BIOB-SH-01):

Time Stamp

- Time of Day Collected:
- Date Collected:

Depth/Elevation

- Elevation at collection site (Meters):

GPS Coordinates:

- Latitude:
- Longitude:

Site Name:

- Country/Ocean, City, Street, Name of business:

Photo

- Take a digital photograph if possible (use your phone):
- Include a metric ruler in the photo so the specimen can be measured

Sample Information

- Life Stage (Adult, Immature, Unknown):
- Sex (Male, Female, Hermaphrodite, Unknown):
- Reproduction (sexual, asexual, Cyclic Parthenogen, Unknown):

Species Identification

- Phylum, Class, Order, Family, Subfamily, Tribe, Genus, Species:

Identification Method

- Barcode, Morphology, etc:

Notes

- Collector's name and any important information:

1.4a. CALCULATING INWOOD HILL PARK INSECT BIODIVERSITY

Objectives: Students will be able to:

1. Identify the number of ***different species*** found in your ecosystem.
2. Identify the number of ***individuals of the same species*** found in your ecosystem.
3. Identify the number of ***different species*** found in Inwood Hill Park.
4. Identify the number of ***individuals of the same species*** found in Inwood Hill Park.
5. Calculate the Simpson's Diversity Index for your ecosystem and Inwood Hill Park.
6. Draw conclusions about their results and how they relate to their original study design.

Part 1: Count up the number of **different species** you have in your ecosystem collection. Use all of the organisms you collected, even if they are not in your immediate collection. For each unique species, count the **number of individuals** you have in your collection.

Example:

Species	**Species Description**	**Number of Individuals (n)**
Species A	Fly with blue eyes, about 2cm	3
Species B	Fly with red eyes, 1cm	2
Species C	Wasp, 4cm	1
Species D	Stink bug	1

Group_____

Species	**Species Description**	**Number of individuals (n)**

Species Richness = total number of species: _____

Total number of individuals of all species (**N**): _____

Part 2: Walk around to the other collections and using the images on the website as well as the physical collection, count up the number of **different species** in **all** of the collections. For each unique species, count the **number of individuals** you have in your collection.

Example:

Species	**Species Description**	**Tally Number of individuals (n)**	**Total Number of Individuals**
Species A	Fly with blue eyes, about 2cm	IIII	4
Species B	Fly with red eyes, 1cm	II	2
Species C	Wasp, 4cm	IIIIII	6
Species D	Stink bug	IIIIIIII	8

Species	**Species Description**	**Tally Number of individuals (n)**	**Total Number of Individuals**

Species Richness = total number of species: _____

Total number of individuals of all species (**N**): _____

Biodiversity background Information

Biological Diversity **-** *the great variety of life*

Biological diversity can be quantified in many different ways. The two main factors taken into account when measuring diversity are richness and evenness.

1. Richness

Richness is a measure of the number of different kinds of organisms present in a particular area. For example, species richness is the total number of different species present in a community. Some communities may be simple enough to allow complete species counts to determine species richness. However, this is often impossible, especially when dealing with insects and other invertebrates, in which case some form of sampling has to be used to estimate species richness.

2. Evenness

Evenness is a measure of the relative abundance of the different species making up the richness of an area. A community dominated by one or two species is considered to be less diverse than one in which several different species have a similar abundance.

Simpson's Diversity Index

Simpson's Diversity Index is a measure of diversity. In ecology, it is often used to quantify the biodiversity of a habitat. It takes into account the number of species present (species richness), as well as the abundance of each species (species evenness). As species richness and evenness increase, so diversity increases.

$$D = \frac{\Sigma\, n(n-1)}{N(N-1)}$$

n = the total number of organisms of a particular species
N = the total number of organisms of all species

The value of **D** ranges between 0 and 1

Simpson's Index of Diversity (I) = 1 - **D**

The value of this index ranges between 0 and almost 1, the greater the value, the greater the sample diversity. The index represents the probability that two individuals randomly selected from a sample will belong to different species.

Part 3: Use the information from Part 1 to calculate the biodiversity of **your ecosystem** using the Simpson's Diversity Index. Use the example below to help you complete the calculation.

Example:

Species	n	n(n-1)
Species A	3	6
Species B	2	2
Species C	1	0
Species D	1	0
Total (N)	7	
∑n(n-1)		8

$$D = \frac{\Sigma\, n(n-1)}{N(N-1)}$$

Show all your calculations here:

Step 1: D = 8/7(7-1)

Step 2: D = 8/42 = 0.19

Step 3: I = 1-D = 1-0.19 = .81

Simpson's Diversity Index = .81

Simpson's Diversity Index Calculation

Species	n	n(n-1)
Total (N)		
∑n(n-1)		

Show all your calculations here:

Part 4: Obtain the Simpson's Diversity Index for each of the ecosystems and fill in the table below.

Simpson's Diversity Index	**Group 1: Upper Ridge Forest**	**Group 2: Field**	**Group 3: Lower Successional Forest**	**Group 4: Lower Ridge Forest**	**Group 5: Beach**	**Group 6: Upper Successional Forest**

Part 5: Use the information from Part 2 to calculate the biodiversity of **Inwood Hill Park** using the Simpson's Diversity Index.

Simpson's Diversity Index Calculation

Species	**n**	**n(n-1)**
Total (N)		
∑n(n-1)		

Show all your calculations here:

Part 6: Use the information from your original study design and your calculations to answer the following questions:

1. Which ecosystem was most diverse? How do you know? Why do you think?
2. Which ecosystem was least diverse? How do you know? Why do you think?
3. What variables may have influenced these results?
4. What were the limitations (potential errors) of your particular study design? How did this affect your data?
5. If you were to do this again, what changes might you make in your study design? Why?
6. How might you use this information to help increase biodiversity at Inwood Hill Park?
7. Based on these results, what new question(s) could you ask for future experiments?

1.5a. INWOOD HILL PARK SCIENTIFIC REPORT

Directions: Use the format below to begin to develop your final paper on Inwood Hill Park Invertebrate Biodiversity. Your final paper should be typed and you must submit one per group.

TITLE: The title should be descriptive and the reader should know what your study is about by reading the title.

1. **INTRODUCTION**:
    - Paragraph 1: Biodiversity – what is it, why it matters, what it does for us.
    - Paragraph 2: Inwood Hill Park History and diversity of ecosystems
    - Paragraph 3: Your ecosystem – talk about your ecosystem, its characteristics and organisms that live there
    - Paragraph 4: Invertebrate diversity and importance to ecosystems
    - Paragraph 5: Purpose of the study, what you expect to find (hypothesis), and why this is an important study.

2. **METHODS**: For each of the bullet points below, explain what you did.

Collection

- Random Sampling
- Site setup (how did you set your plots)
- Collection Methods
- Preservation Methods
- Identification and organization methods (include information on pinning, labeling, and DNA barcoding)
- Are there any visuals you could include that might help the reader understand more about what you did (think about maps, photos, diagrams of traps, etc…)? Describe each below.

Analysis

- Calculating Biodiversity (include information about species richness and Simpson's Diversity Index)

3. **RESULTS**:
    - Visual data – photos
    - Biodiversity data as a bar graph
    - Biodiversity calculation

4. DISCUSSION/CONCLUSIONS
    - Connect your results back to your introduction
    - What are possible errors? How might these have impacted your results?
    - If you were to do this again, how would you improve this study?
    - What new questions can you ask now with this information?

5. **REFERENCES** (APA format)
    - Use citationmachine.net to help you with citations.

1.5b. INWOOD HILL PARK SCIENTIFIC REPORT RUBRIC

Criteria	Points	Comments
Title: (5 points) • Clearly and concisely describes the nature of the study • Includes pertinent information		
Introduction: (35 points) • Provides sufficiently broad background info • Provides rationale (Why is this important?) • Provides a context (What has already been done?) • Goals and objectives of the study are clearly stated • Hypothesis is clearly stated, specific and testable		
Methodology: (40 points) • Methods are directly aimed at testing the stated hypothesis • Methods are feasible • Pertinent diagrams and/or photos included and are informative • Identifies the study area and data collected • Procedures appear to be replicable • Analysis described		
Results: (40 points) • Where appropriate, data are presented in figures (graphs) and tables • Figures and tables correspond with the stated method • Axes, titles and legends of tables and figures are properly labeled • Figures and tables are professional looking and easy to interpret • Appropriate types of figures (line vs. bar) are used • All calculations and observations not in figures and tables are included in the text • Each figure and table presented is described in the text • Figures and tables are cited in the text that describe them • Relevant statistics and statistical analysis are presented • Data and data analysis are presented in a logical order		

Discussion and Conclusions: (50 points) • States whether the hypothesis was supported by the results • Presents a logical explanation and interpretation of the results • Explains the significance of all results • No extraneous information is presented • Describes how these results fit into the "big picture" • Discuss the practical applications of the results • Demonstrates creative and critical thinking • Discusses possible reasons for unexpected results • Identifies and discusses all major potential sources of error • Conclusion paragraph concisely summarizes the paper • Conclusion paragraph restates the major findings • Conclusion paragraph restates the significance of the findings • Conclusion paragraph generates ideas and questions to guide future research		
References: (10 points) • Listed in scientific journal format (APA) • Listed alphabetically		
Organization and Style: (20 points) • Uses headings and subheadings to visually organize the material • Few errors in spelling, punctuation and grammar • All required elements are present and additional elements that add to the paper (e.g., graphs, tables, figures, images) • Research paper handed in on time		
Total Points: (200 points)		

General Comments: