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Life in a Pine Cone

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Middle School Math & Science Collaborative

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This exercise focuses on a little-known microhabitat -- the pine cone. A pine cone's primary function is, of course, reproduction ... housing the seeds of the next generation of conifer trees. However, pine cones are also the basis of a food web that provides both resources and living space for a wide variety of small arthropod species. The procedure outlined below is designed to examine this microhabitat and compare its community diversity among different species of conifers and habitats.

The exercise is based on a 1985 paper, Life in a Pine Cone, by David L. Kulhavy, Robert S. Baldridge and James W. Bing published in *Texas Natural History*. The paper is the basis of a Ward's Bulletin of the same title. The procedures have been modified somewhat in cooperation with D. L. Kulhavy for use in this exercise.

Students, or teams of students, will collect a standard sample of cones from a species of pine tree in a habitat of their choice, extract the arthropods associated with their cones, and provide numerical and descriptive data in a standard format. To ensure comparability between samples, a sampling protocol will be followed.

SAMPLING PROTOCOL

Standardized sampling is essential to allow comparison of data from different students. Although there is considerable flexibility in the choice of sampling site and tree species, procedures within each site and for each species should be consistent with the procedure outlined below.

1. Select a site with several conifer trees bearing cones that can be collected from the ground. Record sample site information on the data sheet provided. The type of data required is shown below. Maps may be used to get latitude and longitude, and perhaps elevation (tie this in with geography!). A local radio station can provide the weather information, or use your own thermometer. Note that percent relative humidity is somewhat difficult to get since many stations now report dew point instead. Examples of different habitat types include: urban woodlot, meadow, conifer forest, mixed forest, alpine area, river edge, ornamental tree, etc.

2. Collect a 2-liter sample of cones from the ground under the sample trees. Place the cones directly into the 2-liter collecting bottle (a 2-liter soda bottle modified to make the bottom removable). Mark the bottle with the basic sample data: name, date, site, and tree species (if known). Store and process the samples separately!

   If you wish to use numeric designations for the month to keep labels short, use Roman numerals for the month and Arabic numerals for the day to avoid confusion (e.g., III-28-1989).

   The sample bottle doubles as a Berlese funnel. Place it, mouth down (cap removed) in a coffee can stand over a baby food jar partially filled a 70% alcohol solution (obtain the alcohol from your lab, or rubbing alcohol works well). As the collected material drys, the arthropods using it as shelter will move down (deeper into the litter) and end up in the alcohol. Allow the samples to sit undisturbed for about one week to drive all specimens in the cones into the collection bottle. Replenish the alcohol as needed because it evaporates rapidly.

3. Process the specimens collected from the sample. Begin by removing sampled material from the bottom of the collecting bottle using an eye dropper and placing it in a petri dish. If the bottle was disturbed in transport, let it settle awhile first.

   Examine the contents of the dish under the microscope, initially under low power, then under a higher power. Spend some time just looking at the variety of organisms in the sample. Become familiar with the variety of species present, noting similarities between specimens that might be the basis for inclusion in the same taxa. It is not necessary to identify specimens taxonomically yet, but the student should develop their own descriptive names for the species they see. Examples might be "golden teardrop," "red spider," "fringed worm," etc. This provides incentive to examine the specimens closely and provides a convenient shorthand for referring to the species when sorting them.

   After initial examination of the material, begin with one of the rare groups of organisms that seem to belong together (e.g. spiders, sowbugs, beetles, etc.) and carefully remove them to one of the empty alcohol vials provided using the eyedropper. [Note: microcentrifuge tubes are a good substitute for vials; placing them in trays cut from plant

seedling trays ensures fewer spills.] Be sure to label the vial with the chosen name for the taxa. The label should be in pencil and go directly into the vial, or directly on the microcentrifuge tube. Lumping of apparent species that obviously belong together into recognizable taxa may be desirable at this point to conserve vials.

Repeat this procedure, leaving the more numerous taxa for last, until all specimens have been sorted into vials. The instructor should probably spot check the vials as they are sorted to provide some early quality control. Clean the petri dish to remove any residual garbage (these samples are initially anything but clean), and repeat the procedure until all material in the collecting bottle has been sorted into vials.

Review the specimens in one of the vials by holding the vial with a thumb over the open end, inverting it over the clean petri dish, letting the material settle a few minutes, and allowing it to flush into the dish. Correct any errors made in the initial sorting. Since the diversity in the dish has been reduced and the garbage removed, this will be easier than during the first sorting.

Count the number of specimens in the dish by moving them to one side using the various manipulators provided, then physically moving each one back to the other side as they are counted. Record the results and transfer them back to their vial using the eyedropper. Include a label with the number of specimens in the vial or on the tube.

Repeat this procedure for each sorted vial. Update the number recorded for previously counted vials if members of that taxa are added during review of subsequent vials, or create new taxa.

4. Review the characteristics of the major taxa found in pine cones (samples should be available for viewing if you have them). Identify the sorted material to using pictorial keys and the examples (if available), then double check their identification with the instructor. While some taxa are easy to identify (e.g., ants and wasps, flies, beetles, isopods, etc.), some are more difficult. Some mites and spiders are easily confused, and springtails, thrips and barklice require some practice.

GROUP RESULTS

Once all sorted material has been identified, consolidate the counts by taxa and enter them on the data sheet provided (ni). At this point you may want to group data for different combinations of tree species and habitat, consolidating data as necessary to make meaningful comparisons. For example, you might combine all white pine samples from urban woodlots for comparison to a spruce sample from a roadside urban habitat. Try to find comparisons that both make ecological sense and that give groups of students of equal size. This also allows you to form groups of students that can reinforce each other as they begin analyzing the data.

The data sheet is designed to easily calculate the proportion (pi) and percent of the sample represented by each taxa. Have the students determine the best statistic for comparing data between groups. That is, the number of individuals is dependent on the size of the sample (the number of standard samples that were combined), but proportion or percent could be used to

compare samples of different sizes. Another approach would be to divide the number of individuals by the sample volume (each standard sample was 2 liters), and report the number of individuals per liter.

Construct relative abundance curves for each habitat, and bar graphs of the abundance of taxa represented in the habitats being compared. For relative abundance, you want to plot the number of taxa represented by 1-10, 11-20, 21-30,. . .,131-140, 141-150, > 150 individuals in the grouped data (there are too few taxa in any single sample to make this graph meaningful). You might try this for the whole class rather than individual groupings. For the abundance of taxa in a specific habitat, plot the number of individuals in the taxa against the taxa number from an individual or combined data sheet.

ADDITIONAL ANALYSES

Advanced students might want to compute a diversity index for different combinations of tree species and habitat, consolidating data as necessary to make meaningful comparisons. The Shannon-Weiner diversity index is the most commonly used:

H' = -SUM(pi*log(pi))

Where: pi= ni/N, N = the total number of individuals in the sample, and ni= the number of individuals in taxa i.

This index (sometimes called the Shannon-Weaver Index) combines the number of species (species richness) with the distribution of individuals among species (equitability) to provide a quantitative measure of diversity in any habitat. The log may be computed using any base, but the base should be consistent within the class. An alternate data sheet is provided that includes columns to aid in the calculation of this index. The larger the index, the greater the diversity in the sample.

EXPANSION OF THE THEME

The investigation of biodiversity on this micro-scale in a local habitat serves as an excellent introduction to the issue of global biodiversity and the destruction of infinitely richer habitats throughout the world, particularly the tropical rainforests. E. O. Wilson's (1992) book, *The Diversity of Life* gives an extensive look at the issues in a style designed for the layperson.

INTEGRATION OF SOCIAL STUDIES AND ART

Certainly the declining biodiversity and its economic causes in, and impacts on, our society, as well as the highly publicized spotted owl controversy in our own Pacific Northwest provide ample opportunity to tie biodiversity into the social studies curricula. Regarding art, there are numerous art works that deal with tropical diversity, but a more interesting approach might be to have the students draw scenes of the perspective of a mite-sized resident of a pine cone community!

Pine Cone Arthropod Diversity Datasheet

Name(s):
Class:
Date:

HABITAT DATA
- Habitat Type:
- Tree Species:
- Elevation (m):
- Latitude:
- Longitude:

WEATHER DATA
- Temperature (deg C):
- % Relative Humidity:
- General Conditions:

SAMPLE DATA
- Date Sampled:
- Sample Size:

TAXA COUNTS	ni	pi	%	Optional--> log(pi)	pi*log(pi)
1. Mites.....					
2. Thrips.....					

3. Springtails.....

4. Ants/Wasps.....

5. Flies.....

6. Beetles.....

7. Crickets/Roaches.....

8. True Bugs.....

9. Centipedes.....

10. Millipedes.....

11. Barklice.....

12. Aphids/Leafhoppers.....

13. Moth/Butterflies.....

14. Spiders.....

Other (List):
15._____.....

16._____.....

17._____.....

18._____.....

19._____.....

20._____.....

TOTAL COUNT N=Sum(ni)= H'= -
SUM(pi*log(pi))=

References

Kulhavy, D. L.;Baldridge, R. S.; Bing, J. W. 1985. Life in a Pine Cone! *Texas Natural History* 1(2):27-28.

Wilson, E. O. 1992. *The Diversity of Life*. Harvard University Press, Cambridge, MA.