Chapter 3

Community Ordination Utilizing Winter Stoneflies

Vinnedge M. Lawrence

Department of Biology
Washington and Jefferson College
Washington, PA 15301

Vinnedge M. Lawrence is a Professor of Biology at Washington and Jefferson College, Washington, Pennsylvania. He received his B.S. (education) and M.A. (zoology) degrees from Miami University and his Ph.D. (entomology) from Purdue Univeristy. In addition to introductory biology, he teaches courses in ecology, entomology, invertebrate zoology, developmental biology, and aquatic biology. His research interests include population dynamics of odonate naids, distribution of winter stoneflies, and breeding behavior of Henslow's sparrow.

COMMUNITY ORDINATION UTILIZING WINTER STONEFLIES

Vinnedge M. Lawrence
Washington and Jefferson College

Objectives. This exercise in community ecology can be conducted in the field in mid-winter when a course offered during the spring term may otherwise be restricted to indoor laboratory exercises. It encourages students to make systematic observations in the field. It combines field and laboratory activity with library research, thereby introducing students to various aspects of scientific endeavor. It involves students in original research without a predictably correct outcome. It uses a computerized key to overcome the resistance exhibited by many students to taxonomic work.

Background. An instructor may be reluctant to take a class into the field for investigations that require interpretation of environmental conditions. It may be impossible to determine those factors that most directly influence the outcome of the investigation. This exercise enables students to obtain data with which they can calculate and graph differences between communities. Each student may then apply observations in accounting for these differences.

Although lack of knowledge of critical environmental factors is not a problem in this exercise, the instructor must locate suitable sites for collecting winter stoneflies. These are insects of the order Plecoptera that are members of the families Taeniopterygidae and Capniidae, although these taxa sometimes are treated as subfamilies of the family Nemouridae. Sites may be located during the winter months by examining bridges that cross streams of good water quality. Peak emergence periods vary with latitude, as Taeniopteryx maura, for example, emerges from mid-December through January in Alabama, from January through March in Virginia, and through mid-April in Nova Scotia. Since bridges are favored emergence sites, locating winter stoneflies should not be difficult in areas with at least a few streams with reasonably good water quality.

Developing a computerized key requires familiarity with the winter stonefly fauna of an area. The key should include every taxon that has been collected, with the possible addition of previously uncollected taxa whose distributions overlap the region of the study. A good way to accomplish this is to engage one or more students in independent studies to catalog the local fauna. My experience suggests that an area limited by easy access for field trips, which may be a circle with a 15-mile radius, will include a manageable number of species that will not overwhelm students unfamiliar with stonefly taxonomy. The number probably will be small enough to allow you to include several additional taxa in your key. The key used in this workshop includes four species that definitely occur at my study sites and four others that have not been found or confirmed in the area. Stoneflies neither bite nor sting, and have no characteristics that most students would find objectionable. Many of my students are fascinated to find adult insects in winter in places so accessible, but which they have overlooked.

Procedure. Depending on the instructor's preference, this exercise may require from two to five three-hour laboratory periods. Assuming that five periods are devoted to the project, the first session would occur in the laboratory, followed by two periods of gathering data in the field, after which a fourth period is alloted for identification, and the fifth is used to explain statistical procedures and other details of format to be followed in writing the report of the exercise.

Period 1. I introduce the recording barometer in the laboratory, the sling psychrometer, and Hach Chemical Co. kits for determining pH, dissolved oxygen, nitrate, nitrite, and orthophosphate. After orientation in the correct use of this equipment, each student obtains physical and chemical data in a "dry run" of the procedures each will follow in the field. The barograph is read, relative humidity is determined outside, and prepared water samples arc analyzed. Over the next two weeks, each student will be required to carry out some or all of these procedures at one or more sites in the field.

Period 2. The class travels to an area where a stream system affords at least four bridges that will serve as collecting sites which can be sampled during the period. The sites should be at different tributaries or streams of different sizes. At each bridge, one student obtains physical and chemical data while all other students collect stoneflies from the structure and its immediate vicinity during a 10-minute period. Stoneflies are collected with forceps and placed in homeopathic lip vials that are half filled with 70% ethanol and contain labels indicating the collector's name, date, and collection site. At the end of the collecting interval, a green neoprene stopper is inserted in the vial with the aid of a bent paper clip to prevent pressure buildup from trapped air that could subsequently loosen the stopper and permit evaporation of contents. Screwcap vials, although more convenient in the field, introduce a high probability of evaporation and resultant destruction of specimens.

Following the collecting interval, time is allotted for taking notes on characteristics of the site. These may include estimates of cloud cover, extent of canopy cover when leaves would be present, abundance of leaf packs in the water, substrate type in terms of particle size, extent and proximity of riffles upstream of the site, presence and location of tributaries, size of stream above the site, time of collection, and other pertinent information. By now the student gathering physical and chemical data has completed this activity. This includes obtaining water and air temperatures, relative humidity, pH, a water sample fixed for subsequent dissolved oxygen titration, and a separate water sample for subsequent determination of nitrate, nitrite, and orthophosphate. When we return to the laboratory, we determine the chemical characteristics of the water samples and read the barometric pressure that has been recorded for each of the four collecting intervals.

Period 3. We carry out the same procedures as in the second period, but on a different stream system. The addition of these four rites brings to eight the number of sites we will compare on the basis of their winter stonefly communities.

Period 4. Although students may begin identifying their specimens as soon as they have collected them, I usually devote a laboratory period to assisting them in this endeavor. Students who have already completed their determinations nay wish to reconsider some of them. Those who haven't finished are now imbued with a sense of urgency to do so. Each student is required to submit all stoneflies in their proper vials along with a determination sheet on which the sex and species of each specimen are indicated. I make spot checks for accuracy and examine the entire contents of a collection if I detect errors. Students receive credit for their collections based upon the total number of specimens and the number properly identified. There may be 50 points for the collection, with the number of misidentifications subtracted from the number of specimens submitted, ttie number properly identified, or some other formula that weighs both collecting effort and accuracy of identification.

Since each workshop participant will identify the stoneflies in a sample vial, here are some characteristics to watch for in making your determinations.

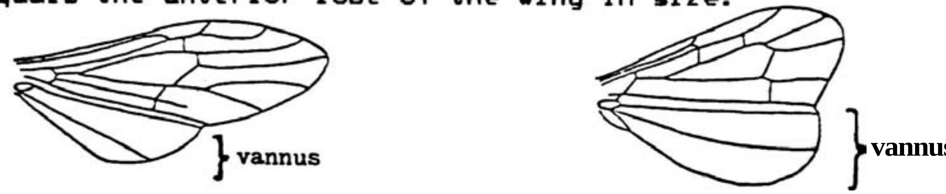
1. The last segment of the leg of an insect is the <u>tarsus</u>. In stoneflies, ttie tarsus is divided into three subsegments known as <u>tarsomeres</u>. Carefully compare the relative lengths of the tarsomeres on a leg. In the family Taeniopterygidae, the 2nd tarsomere is subequal to (about as long as) the 1st or the 3rd. In the Capniidae, the 2nd tarsomere is much shorter than the 1st or 3rd. Since capniids are called small winter stoneflies, you know two characteristics that distinguish them from taeniopterygids.

2. The last abdominal segment of many insects bears a pair of appendages called cerci. The cerci of stoneflies tend to be long and filamentous, but are reduced in Taeniopterygidae where they also exhibit sexual dimorphism. Taeniopteryx females have short, six-segmented cerci, whereas the cerci are represented by a single, inconspicuous segment in males.

3. The cerci of Capniidae are long, but sexual dimorphism is evident in the recurved, <u>supra-anal process</u> of the male that is absent in the female. The supra-anal process recurves anteriorly over the 9th and 10th abdominal segments. It consists of a single element in the genera Nemocapnia and Paracapnia and in Capnia vernalis. In other species of Capnia and in the genus Aliocapnia, it is comprised of two distinct elements, one dorsal and one ventral.

4. The hind wings of capniid stoneflies exhibit a marked dichotomy with respect to the extent of the posterior, flexible lobe, known as the vannal lobe or vannus. In the getiera Capnia, Nemocapnia, and Paracapnia, the vannus is of normal size (Fig. 1), whereas it is exceptionally

large in members of the genus *Allocapnia* in which it equals the anterior lobe of the wing in size.



Paracapnia **Allocapnia**

Figure 1: Hind wings of *Paracapnia* and *Allocapnia.*

With these characteristics in mind, obtain a vial of stoneflies and a Syracuse watch glass or culture dish partially filled with water. Locate your stonefly identification sheet. Record the date and site from the label in the vial onto your identification sheet (the site is recorded by circling the appropriate number). Remove the stoneflies from the vial with forceps and place them in the water. Place the watch glass or culture dish on the stage of a stereomicroscope and adjust the illuminator. Insert a minidisk labeled "Stoneflies" into the disk drive and plug in the microcomputer and monitor. When asked for the program you want, type in "Stoneflies" and press the "RETURN" key. When the program menu display appears, select the dichotomous key by typing in "K". The program will begin illustrating characteristics and requesting yes (Y) or no (N) responses. Type in only the appropriate letter. Use a pair of teasing needles to position a specimen so as to determine the characteristic being requested. Continue until the program indicates the species and sex of the specimen. Record the species name on the identification sheet and place a hash mark in the space corresponding to the proper site and sex. Return this specimen to the vial and proceed to determine another specimen. Continue this procedure until you have identified each specimen. When all specimens are back in the vial, insert the straightened end of a paper clip along with the neoprene stopper into the mouth of the vial. When the stopper has been twisted in securely, hold it in place with your thumb and withdraw the paper clip. Follow the same procedure with any other vial for which you are responsible for determining the contents. Now transfer your data to another identification form on which the totals of the hash marks are entered as numbers. Submit this identification form so that your data are included with those obtained by other members of the group. Pour the water from your otherwise empty watch glass or culture dish and dry it with a paper towel. You may wish to attempt the quiz or examine other parts of the STONEFLIES program while the group results are being tabulated.

Period 5. We now proceed to analyze our stonefly data by constructing a community ordination to compare collection sites. Each bridge is treated as a community and analyzed for its degree of similarity or dissimilarity to every other bridge on the basis of their respective stonefly faunas.

For the sites whose fauna you helped determine, find the frequency for each species. The frequency, in this context, is the percentage of samples obtained in which a particular species

Name ________________________

	Date										
Species	Site	1	2	3	4	5	6	7	8	9	10
	♂										
	♀										
	♂										
	♀										
	♂										
	♀										
	♂										
	♀										
	♂										
	♀										
	♂										
	♀										
	♂										
	♀										

Name ________________________

	Date										
Species	Site	1	2	3	4	5	6	7	8	9	10
	♂										
	♀										
	♂										
	♀										
	♂										
	♀										
	♂										
	♀										
	♂										
	♀										
	♂										
	♀										
	♂										
	♀										

Name ________________________

	Date										
Species	Site	1	2	3	4	5	6	7	8	9	10
	♂										
	♀										
	♂										
	♀										
	♂										
	♀										
	♂										
	♀										
	♂										
	♀										
	♂										
	♀										
	♂										
	♀										

occurred. Now find the number of individuals of this species in all samples combined, and record this as the density of the species. Calculate a prominence value for each species by multiplying its density by the square root of its frequency. Sum the prominence values for all species found at the bridge. This information must be determined for every site.

Construct a matrix of similarity by filling in the portion of the chart on Form 1 above and to the right of the diagonal. The value to be placed in each block is the coefficient of similarity between the two sites that intersect at the block. The coefficient of similarity (C) is calculated by the formula:

$$C = \frac{2w}{a+b},$$

where a = the sum of the prominence values for one bridge? b = the sum of the prominence values at the other bridge, and w = the sum of the lower prominence values of species common to both bridges. Assume, for example, that species X, Y, and Z occur at bridges A and B. Their prominence values at Bridge A are X = 10, Y = 63, and Z = 1. At Bridge B, X = 1, Y = 33, and Z = 36. To find w, the lowest values for each species common to the two bridges are summed; thus w = 1+33+1, or 35. The coefficient of similarity for these bridges would be:

$$C = \frac{2 \times 35}{74 + 70}$$

or .486, a value near the middle of the similarity index which ranges from 0.000, if no species is common to both bridges, to 1.000 when every species occurs at both bridges with the same numbers of individuals present at each. Place your results in the proper spaces of the similarity matrix of Form 1.

It is necessary to convert similarity coefficients to values expressing dissimilarity because the distances between communities, as they will appear on the ordination graph (Form 2), represent degrees of difference rather than similarity. Dissimilarity values are obtained by subtracting the calculated coefficient of similarity for each community from its theoretical maximum similarity value of 1.000. Place these results in the proper spaces of the matrix of dissimilarity of Form 1.

To determine the two most dissimilar communities for placement on the x-axis of the ordination graph, sum the dissimilarity values associated with each bridge. List each community and the sum of its dissimilarity values in the chart at the bottom of Form 1. The bridge with the greatest dissimilarity sum is assigned a value of zero and placed at the left end of the x-axis. Actual placement of points on the graph must await determination of values for the y-axis. Do not begin your graph until the values of all coordinates have been determined. Identify the bridge located at zero on the x-axis as bridge "a", and refer to the matrix of dissimilarity for the bridge exhibiting the greatest dissimilarity to it. This bridge, designated "b", will be placed at the opposite end of the x-axis, and the length of the x-axis will be made equal to the dissimilarity of the two reference bridges. The distance (x) of each of the remaining bridges from bridge "a" along the x-axis is obtained from the formula:

$$x = \frac{L^2 + D_a{}^2 - D_b{}^2}{2L}$$

where L = the distance between the two reference bridges (or dissimilarity value between bridges "a" and "b"), Da = dissimilarity value between bridge "a" and the bridge being plotted, and Db = dissimilarity value between bridge "b" and the bridge being plotted. Record these values in the appropriate column in the chart.

Even after the dissimilarities between bridges have been plotted on the x-axis, some communities that may be quite dissimilar could still be placed close together. Their placement on the y-axis coordinate should be accomplished in such a manner that the maximum component of the remaining dissimilarity is spread along the y-axis. To achieve this, the bridge with the poorest fit on the x-axis must first be determined. A poorness of fit value (e) is calculated for each bridge by the formula:

$$e = \sqrt{D_a{}^2 - x^2} \text{ .}$$

Record your calculated e values in the appropriate column of the chart. The bridge exhibiting the greatest e value is designated a' and assigned a value of zero on the y-axis. The bridge with the greatest dissimilarity to a' and located within 1/10 L of a' on the x-axis is designated b' and placed at the opposite end of the y-axis. The 1/10 L restriction may necessitate a compromise in that any bridge located so near a' on the x-axis may not exhibit much dissimilarity to it. Even though this may be the case, disregarding the 1/10 L restriction could result in the x-axis merely being tilted on the graph, thus negating any true 2nd dimension that the y-axis may otherwise have conferred. When the two reference bridges, a' and b', have been selected, the length of the y-axis is made equal to their dissimilarity.

The distance (y) of each of the remaining bridges from bridge a' on the y-axis is obtained from the formula:

$$y = \frac{(L')^2 + (D_a')^2 - (D_b')^2}{2L'} \text{ .}$$

where L' = the dissimilarity value between bridges a' and b', etc. Record these values in the appropriate column in the chart. The positions of the communities may now be plotted on Form 2 which should consist of a two-dimensional graph constructed on regular graph paper. The graph should be entitled "Two-dimensional ordination of communities on the basis of prominence values of winter stoneflies."

The <u>ordination interval</u>, or direct distance between any two communities in the ordination graph, may be calculated by the formula:

$$\text{ordination interval} = \sqrt{dx^2 + dy^2} \text{ ,}$$

where dx = distance between communities on the x-axis and dy = distance between communities on the y-axis. The extent to which the spacing of the communities on the two-axis ordination accounts for

Date______________ FORM 1 Name_______________________

Similarity and Dissimilarity Coefficients Between Communities

Matrix of Similarity

Matrix of Dissimilarity

	1	2	3	4	5	6	7	8	9
1									
2									
3									
4									
5									
6									
7									
8									
9									

Community Dissimilarity Sums, e Values, and Ordination Coordinates

Community	Sum of Dissimilarity Values	Distance From Community a along x-axis (x)	Poorness of Fit on x-axis (e)	Distance From Community a' along y-axis (y)

the calculated dissimilarities in community composition may be estimated by correlating ordination intervals with dissimilarity values for at least 20 random1y selected community pairs. If the ordination interval is designated X and the dissimilarity value Y, the correlation coefficient (r) is determined by the formula:

$$r = \frac{\Sigma XY - \frac{(\Sigma X)(\Sigma Y)}{N}}{\sqrt{\left[\Sigma X^2 - \frac{(\Sigma X)^2}{N}\right]\left[\Sigma Y^2 - \frac{(\Sigma Y)^2}{N}\right]}}$$

where N = number of community pairs used in making analysis. For this analysis, 20 sets of values should be chosen with the use of a table of random numbers and entered in Form 3. The higher the correlation coefficient, the more satisfactorily the two-axis ordination accounts for the calculated dissimilarites between communities. A correlation coefficient above 0.90 indicates a very satisfactory accounting.

Discussion. One of the values of the ordination graph is that it may help indicate those environmental factors that are most important in influencing community composition. This is illustrated by Finni (1973) for winter stonefly communities in Little Pine Creek during the winters of 1967-68 and 1968-69. After the ordination has been constructed, environmental gradients corresponding to the x- and y-axis variables should be sought and plotted along these axes. The number of stoneflies collected at a site may be influenced by air temperature and amount of insolation at the site during and prior to the collecting interval. Stonefly abundance at a site may be influenced by the number and size of leaf packs upstream. Prevalence of leaf packs may, in turn, reflect extent of riffle areas to catch leaves and extent of canopy cover in summer to provide leaves. The occurrence of diapause in the life history of a species may influence its presence at a site. Permanent streams can support species that are absent from streams that dry up periodically. Species whose nymphs undergo diapause in the hyporheic zone may be particularly abundant in intermittent streams where competition from non-diapausing species is lacking. Small, spring-fed tributaries may support populations of small, non-diapausing species that require very little flowing water, but must be in water throughout their development.

This exercise should include the requirement of a laboratory report written in a style reflecting proper format for scientific writing. Helpful suggestions are provided by Brower and Zar (1984), who have produced a very useful ancillary text for ecology courses that includes a table of random numbers, common statistical procedures and tables, and detailed methodologies for various ecological investigations.

Literature Cited

Brower, J. E., and J. H. Zar. 1984. Field and laboratory methods for general ecology. 2nd ed. Wm. C. Brown: Dubuque, Iowa.

Finni, G. R. 1973. Biology of winter stoneflies in a central Indiana stream (Plecoptera). Ann. Entomol. Soc. Am. 66:1243-1248

Date________________ FORM 3 Name____________________________

Calculation of Correlation Coefficient Between Ordination Interval and Community Dissimilarity Values

Show equations with actual numerical values as terms are reduced:

Community Pair	Ordination Interval (X)	Dissimilarity Value (Y)	XY	X^2	Y^2
N = 20	$\Sigma X =$ $(\Sigma X)^2 =$	$\Sigma Y =$ $(\Sigma Y)^2 =$	$\Sigma XY =$	$\Sigma X^2 =$	$\Sigma Y^2 =$
		r = ________			

Sources of Materials

Hach Company
P. O. Box 389 (800) 525-5940
Loveland, CO 80539

Cat. No.	Item
1802-02	Water Ecology Test Kit, Model AL-36B
1475-00	Orthophosphate Test Kit, Model PO-14
14081-00	Nitrate-Nitrite Test Kit, Model NI-12

VWR Scientific
P.O. Box 8188 (800) 257-8407
Philadelphia, PA 19101

Cat. No.	Item
59589-110	Green Neoprene Stoppers, Size 0
66010-129	Homeopathic Lip Vials, 3 dram
66010-140	Homeopathic Lip Vials, 4 dram

WeatherMeasure Corporation
P.O. Box 41257 (916) 481-7565
Sacramento. CA 95841

Model No.	Item
HM 10	Sling Psychrometer
B201-S	Barograph with spring-wound clock

Acknowledgement

I greatly appreciate the contributions of Dr. Thomas Hart (Department of Biology, Washington and Jefferson College) and Dr. Rick Peifer to the preparation of this workshop. Tom wrote the computer program STONEFLIES to provide my students with an alternative to the other taxonomic keys I provide in my course. Rick was most helpful in gathering the equipment and supplies to run the workshop.