

# <sup>1</sup>MODULE 6: PREDATOR FEEDING BEHAVIOR

## Introduction

Predators are often selective in the prey that they consume, focusing on particular types (mobile versus immobile) or sizes of prey. Predators often also feed at particular times of day or change feeding patterns with the seasons. This is particularly true for stoneflies, who appear to feed under diminishing light conditions that occur at dusk or in the dark. [Why would they do this since they are not visual predators themselves?] In addition, stoneflies are often size selective predators—larger stoneflies consume larger prey than smaller stoneflies. In this laboratory we will examine differences in feeding behavior of stoneflies by examining the contents of the stomachs of stoneflies either collected during two different time periods or two different sizes of stoneflies collected at the same time. This exercise is modified from Peckarsky (1996).

The most common way of measuring patterns of differential predation is to compare the prey remains found in predators guts. Although gut content data may provide an accurate record of undigested prey parts, there are many potential limitations to this method. Differential gut clearance time of different prey species may lead to overestimation of prey with heavily sclerotized parts compared to soft bodied prey. Partial consumption of prey may leave heavily sclerotized parts uneaten. Furthermore, ingestion of prey fragments or prey maceration may also constrain our ability to identify and quantify predator diets from gut contents. Also, regurgitation may occur during preservation, or preservatives may alter gut contents. Thus, gut contents show only part of what has been eaten and could result in misinterpretation of the relative consumption of different prey species. Gut contents do not give any information on the availability of prey species.

## Methods

**Protocol for Stomach Content Analyses:** Use two pair of forceps to pull the head from the prothorax of individual stoneflies (be sure to select only the predatory ones). The foregut, which should remain intact and attached to the head, can then be dissected and examined for recognizable prey parts. If the foregut does not remain attached to the head, dissect the thorax (through the ventral side) and anterior abdomen to extract the foregut. Since large, predatory stoneflies swallow their prey whole, prey should be identifiable, provided a short time has elapsed since the predators last meal (see problems with this method as discussed above). Stonefly guts will also often contain detritus and algae (diatoms) and may even be empty. Use the keys and books provided to identify prey in the predator's foregut. Stoneflies primarily eat dipterans (midges and black flies), mayflies and caseless caddisflies. If

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prey are fragmented, compare fragments (claws, mandibles, head capsules, etc) to pictures of whole specimens or the specimens we collected in our previous survey laboratory. Be sure to note the time period when the stoneflies were collected at the top of the data sheet. For each stonefly, enter the data on Data Table 1. Indicate the presence or absence of detritus and diatoms and if the gut was empty. Indicate the number of each prey type and the total number of prey found in each gut. If needed, extra copies of Data Table 1 will be available in class.

**Data Analysis:** Combine data from the entire class on Master Data Tables 2, 3 and 4. For each table, each stonefly can only appear in one cell (only in one spot on the table). We will undertake analyses to show whether feeding habits of the stoneflies vary between the two time periods. Significant variation between these time periods will be indicated by a Chi Square ( $\chi^2$ ) Test of Independence. If significant variation is found, it would suggest that stoneflies exhibit diel feeding periodicity. These same tests will tell us whether stonefly guts are dominated by one food type (Master Data Table 2), whether stoneflies tend to differ in the amount of prey in their guts between time periods (Master Data Table 3), and whether stonefly guts are dominated by a particular animal food type (Master Data Table 4).

The Chi Square Test of Independence is a very simple test to do. What we do is compare the actual number of individuals in a category (called “**observed**”, **O**, or **OBS**) to the value expected (called “**expected**”, or **E** or **EXP**) under some hypothesis (this can vary depending on the test). In this case we will test the hypothesis that the two time periods do not differ. Master Data Tables 2, 3, and 4 are set up to do the Chi Square test; however, you may need to modify the Tables. Situations under which modifications need to be made will be explained below. To do the test of independence your tables need to be set up like the one below (this is true for any test you may want to do):

CATEGORIES OF A	CATEGORIES OF B				TOTAL
	B <sub>1</sub>	B <sub>2</sub>	.....	B <sub>c</sub>	
A <sub>1</sub>	n <sub>11</sub>	n <sub>12</sub>	.....	n <sub>1c</sub>	n <sub>1.</sub>
A <sub>2</sub>	n <sub>21</sub>	n <sub>22</sub>	.....	n <sub>2c</sub>	n <sub>2.</sub>
..	..	..	.....		
..	..	..	.....		
..	..	..	.....		
A <sub>r</sub>	n <sub>r1</sub>	n <sub>r2</sub>	.....	n <sub>rc</sub>	n <sub>r.</sub>
TOTAL	n <sub>.1</sub>	n <sub>.2</sub>		n <sub>.c</sub>	n

This table is called an r x c contingency table because it has c number of columns (the subscript on B) indicating the number of categories of the B variable and r number of rows (the subscript of A) indicating the number of categories of the A variable. The entries in the table ( $n_{rc}$ ) are the frequencies (counts of things) in that particular combination of categories. The  $n_{.c}$  are the sums of the frequencies of a single row (across the columns) and the  $n_{.r}$  are the sums of the frequencies of a single column (across the rows). The total of all rows and columns is n. Notice this table is set up in the same manner as Master Data Tables 2, 3, and 4.

To calculate the Chi Square statistic ( $\chi^2$ ) you must determine the expected value (E) for each cell. If we use the index i for the row subscript (r) and j for the column subscript (c) then the expected value for each cell is

$$E_{ij} = (n_{i.}n_{.j})/n.$$

The Chi Square statistic is written

$$\chi^2 = \sum_{i=1}^r \sum_{j=1}^c (O_{ij} - E_{ij})^2 / E_{ij}$$

where  $O_{ij}$  is the observed frequency in cell ij and  $E_{ij}$  is the expected frequency for cell ij.

Once calculated this statistic is compared to a Chi square distribution with  $f = (r - 1)(c - 1)$  degrees of freedom. You also have to decide on an  $\alpha$  value to use (usually 0.05). If the calculated Chi square value is higher than the value in the table of Chi square values then there are significant differences between the two time periods.  $\chi^2$  tables will be available in class.

As I mentioned earlier you may have to redo the tables before you do the statistics because of restrictions of the values in the table which has to do with the assumptions behind the chi square statistic. First, if any row (across all the columns) or any column (across all the rows) has all zero entries delete it and draw a new table. Second, if any value in a cell ( $n_{ij}$ ) is less than 5, then combine rows. For example, if Diptera only show up once and Plecoptera show up 7 times combine them into a category called Diptera & Plecoptera which now has 8 entries.

## Literature Cited

Peckarsky, B.L. 1996. Predator-prey interactions. Pp. 431-451 In Hauer, F.R. and Lamberti, G.A. (Eds.) *Methods in Stream Ecology*. Academic Press, NY.

## **Assignment:**

**Write a short two page summary of your results. Remember that we may change the chi-squared tables in class based on our results. The specific part of each question to be answered will be determined by which question (diel periodicity or stonefly size) we examine in the laboratory. Be sure to answer the following questions in your summary and turn in with your data sheets:**

1. Do stoneflies exhibit temporal feeding periodicity or do the two sizes of stoneflies have different numbers of empty guts? [Compare empty guts versus others in Master Data Table 2 and number of items in gut in Master Data Table 3 between time periods.]
2. What animal items appear most frequently in the gut? Do these change between time periods or between the large and small stoneflies? [Compare items in guts in Master Data Table 4 between time periods.]
3. Does information on gut contents tell you whether the stoneflies “preferred” a prey type?

# DATA TABLE 1

Time collected:

Period:

TAXON	STONEFLY									
	1	2	3	4	5	6	7	8	9	10
Detritus (P/A)										
Algae (P/A)										
Ephemeroptera (mayflies) (number)										
Plecoptera (stoneflies) (number)										
Trichoptera (caddisflies) (number)										
Diptera (midges, blackflies and other fly larvae) (number)										
Other Organisms (number)										
Gut empty										
Total animals in gut										

## MASTER DATA TABLE 2

FOOD CATEGORY	TIME PERIOD OR SIZE OF STONEFLY				TOTAL NUMBER (OBS)
	1-		2-		
	OBS	EXP	OBS	EXP	
Gut empty					
Algae only					
Detritus only					
Animal only					
Algae & Detritus					
Algae & Animal					
Detritus & Animal					
Detritus, Algae and Animal					
TOTAL NUMBER (OBS)					

### MASTER DATA TABLE 3

NUMBER OF PREY IN GUT	TIME PERIOD OR SIZE OF STONEFLY				TOTAL NUMBER (OBS)
	1-		2-		
	OBS	EXP	OBS	EXP	
0					
1					
2					
3					
> = 4					
<b>TOTAL NUMBER (OBS)</b>					

# MASTER DATA TABLE 4

(ONLY THOSE WITH ANIMALS IN GUT)

<b>PREY CATEGORY PRESENT IN GUT</b>	<b>TIME PERIOD OR SIZE OF STONEFLY</b>				<b>TOTAL NUMBER (OBS)</b>
	1-		2-		
	OBS	EXP	OBS	EXP	
<b>Ephemeroptera (mayflies)</b>					
<b>Plecoptera (stoneflies)</b>					
<b>Trichoptera (caddisflies)</b>					
<b>Diptera (midges, black flies and other fly larvae)</b>					
<b>Other Organisms</b>					
<b>More than one type</b>					
<b>TOTAL NUMBER (OBS)</b>					