PREDATION AND PREY COMMUNITY STRUCTURE:
AN EXPERIMENTAL STUDY OF THE EFFECT OF MOSQUITO
LARVAE ON THE PROTOZOA COMMUNITIES
OF PITCHER PLANTS

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Abstract. This work was designed to test the hypothesis: in the presence of predation more species will coexist in the prey community than in the absence of predation. The hypothesis may also be stated in terms of the evenness with which individuals are distributed among species.

This hypothesis was tested using the communities that occur within the water-filled pitchers of the northern pitcher plant, Sarracenia purpurea L. The predator in this system is the larval stage of the mosquito, Wyeomyia smithii (Coq.). The prey community consisted of ciliated and flagellated protozoans as well as rotifers.

Four approaches were used to gain information about this predator-prey system: (1) observations on the densities of the predator in the field, (2) observations on the correlation between predator density and protozoan community structure in the field, (3) field experiments in which both the predator density and initial protozoan community structure were controlled, and (4) observations on the development of protozoan community structure in the long-term absence of predation.

The results show that there is considerable variability of predator densities in both time and space and that the protozoan community responds to these differences. The field experiments show that for the pitcher plant system the hypothesis must be rejected: species numbers tend to drop monotonically as predator densities increase. On the other hand, evenness (H/H\text{max}) does increase as predator densities increase. These patterns hold except during diapause of the mosquito larvae in the fall.

These results are discussed within a general framework of factors which appear to be important for determining how predators affect prey community structure; the four possible mechanisms by which species numbers could increase with predation are outlined, and the pitcher plant system is related to each possible mechanism.

The pitcher plant system has very few characteristics that would cause the system to respond to predation with enhanced species numbers. The most important characteristic leading to decreased species numbers appears to be a low level of interaction among the protozoan species. The increase in evenness in response to predation can best be explained by a shift in the factors that control species numbers from species interactions to rates of reproduction.

Key words: Community structure; diversity; Paine's hypothesis; pitcher plants; predation; protozoans.

INTRODUCTION

A variety of mechanisms have been proposed to explain the composition and structure of communities at the local level. Four of these appear to be most significant: the intensity and pattern of both competition (Levins 1968, Vandermeer 1970) and predation (Paine 1966, 1971, J. Harper 1969), spatial heterogeneity (MacArthur and Levins 1964, Levin 1970), and temporal heterogeneity (Hutchinson 1961). These four factors form an interacting set, such that the relative importance of any one of the factors will depend upon the values for the others; and the assessment of relative importance of these four will also depend upon the particular aspects of the community being studied—dynamics, structure, or species composition. For this study I am restricting myself to a consideration of how the intensity of predation affects prey-community structure, where structure is assessed primarily by the number of species in the community and by the evenness with which individuals are distributed among these species.

There are numerous examples from the ecological, agronomic, range management, and biological control literature demonstrating the potency of predators (including grazers), both as they affect individual species and the community as a whole. The structure of plant communities can be controlled through man's control of large herbivores (e.g., Jones 1933), through the introduction of plant predators (e.g., Huffaker and Kennet 1959), and in more natural systems by the actions of small mammals (e.g., Batzli

The effect of predators on prey-community structure has been considered theoretically. Two species competition may be stabilized by the actions of a predator, as has been argued theoretically by Slobodkin (1961), Parrish and SAILA (1970), Hutchinson (1961), Cramer and May (1972) and others, and as has been demonstrated by Slobodkin (1964), Utida (1953), Park (1955) and Connell (1961). These arguments generally depend upon either selectivity by the predator on the best competitor, or, with nonspecific predation, on a higher reproductive rate by the inferior competitor. In multispecies systems predation may increase the number of species in the community as has been observed by Paine (1966, 1971) for intertidal communities and as has been inferred for plant communities by K. Harper (pers. comm.) and B. Bartholomew (pers. comm.).

The present working hypothesis concerning the effect of predation on the number of species in the prey community derives from Paine's (1966) statement that "local species diversity is directly related to the efficiency with which predators prevent the monopolization of the major environmental requisites by one species." That is, under certain circumstances there may be more species in the prey community when the predator is present than when it is absent.

It appears that this may result under two general sets of conditions. In the first we may consider systems where there is a continual disequilibrium between the predator and prey populations. For example, in systems where the prey community is statically structured in space and the predator removes its prey from the environment, predation establishes open spaces in the community which may be invaded by new individuals; if the invading individual is not of the species that normally dominates competitively then the effect will be increased species numbers. In short, the predator acts by creating sets of microsuccessional sequences separated in space and time. When the entire range of the predator is considered, the effect is an increase of species in the community (Paine and Vadas 1969).

Equilibrium between the predator and prey populations could bring about increased species numbers in the following manner. In the absence of predation or where predator densities are very low, the effects of competition should lead to a community from which a number of species that could otherwise exist in that habitat are excluded. Under conditions of intermediate predation intensity, however, the densities of species already present would be lowered, thereby increasing the probability of successful invasion of the community by species previously excluded; at higher predation intensities the capacities for increase of many or most of the species may be exceeded, resulting in an even lower number of species than was present without predation.

This general pattern of community response is shown in Fig. 1a. It is, of course, also possible that predation will simply result in the loss of species from the community, the loss being greater the higher the predator density. These two patterns will be referred to as enhanced diversity and decreased diversity, respectively, and they are presented in general form in Fig. 1.

The purpose of my experimental field work was to determine unequivocally which pattern of species response occurs in the protozoan communities of the northern pitcher plant, Sarracenia purpurea L., where the protozoans are exposed to predation from the larvae of the mosquito, Wyeomyia smithii (Coq.). My hypothesis was that the system would respond with increased species numbers as predation increased. Such a result would strongly support a generalization of Paine's hypothesis, as the pitcher plant system is markedly different from the intertidal both in its physical structure and in the mechanisms of interactions between predator and prey and among prey.

Observations by others who have worked on the
protozoan communities in small aquatic container habitats (Maguire et al. 1968, Kurihara 1957a, b, 1958) indicate that high predator densities can lead to the almost complete removal of the protozoan community, but their data do not permit an interpretation of how species numbers respond at lower predator densities. Preliminary experiments on the pitcher plant system showed that enhanced diversity could occur, and that more thorough observations might demonstrate this as a regularly predictable result.

Four approaches were used for the solution of this problem: (1) observations of the densities of the predator in the field, (2) observations on the correlation between predator density and protozoan-community structure in the field, (3) field experiments in which both the predator density and initial protozoan-community structure were controlled, and (4) observations on the development of protozoan-community structure in the long-term absence of predation.

**Materials and Methods**

*Description of pitcher plant system*

The northern pitcher plant, *Sarracenia purpurea* L. is locally abundant and distributed from the Gulf of Mexico to the Northwest Territories of Canada (Wherry 1933). It has most of the desirable features of container habitats: (1) the leaves are functionally isolated from each other, (2) they are abundant, (3) the fauna and the predator-prey system are simple, (4) both the prey and the predator populations can be easily observed and manipulated, and (5) the density of predators varies greatly in both time and space. The leaves of *Sarracenia purpurea* form the experimental units for my study, and each leaf appears to be independent with respect to the effects of the predator on the prey community.

The larval *Wyomyia smithii* constitute the only consistent source of predation upon the protozoan and rotifer community of the pitchers; other pitcher inhabitants either have inappropriate feeding habits or are too rare to be important sources of predation upon the protozoans. Many species of mosquito larvae are effective filter feeders upon protozoans (Bates 1949, Hinman 1930, Maguire et al. 1968) and my own laboratory observations confirm this for *Wyomyia smithii*.

The prey organisms for the mosquito larvae are ciliate and flagellate protozoans as well as small rotifers. Table 1 gives a list of the taxa recognized during the course of my study: the size range is between 10 μ for the small *Monas* species and 100 μ for the rotifers. Interestingly, the taxa which I encountered are very similar to those which Kurihara

<table>
<thead>
<tr>
<th>Table 1. Protozoan and rotifer taxa used during the study</th>
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<tr>
<td><strong>Flagellates</strong></td>
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<tr>
<td>Chrysomonadida</td>
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<td>Monas vulgaris</td>
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<td>Chilomonas paramecium</td>
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<td>Astasia klebsi</td>
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<td>Scytomonas pusilla</td>
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<td>Anisonema emarginatum</td>
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<td>Notozoon sp.</td>
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<td><strong>Volvocida</strong></td>
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<td>Chlamydomonas sp.</td>
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<td>Chlorogonium sp.</td>
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<td>Carteria sp.</td>
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<td>Polysoma agilis</td>
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<td><strong>Kinetoplastida</strong></td>
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<td>Bodo sp.</td>
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<td>Cercocones sp.</td>
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<td><strong>Trichomonadida</strong></td>
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<td>Urophagus rostratus</td>
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<td>Ciliates</td>
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<td><strong>Gymnostomatida</strong></td>
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<td>Urotira ovata</td>
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<td>Urotira agilis</td>
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<td>Platyphrya spumacola</td>
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<td><strong>Trichostomatida</strong></td>
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<td>Colpoda inflata</td>
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<td>Colpoda sp.</td>
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<td>Bressiauna sp.</td>
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<td>Lepifiophrya splagnosorum</td>
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<td>unknown microthoracid</td>
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<td><strong>Hymenostomatida</strong></td>
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<td>Colpida campyllum</td>
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<td>Pseudoglaucoma muscorum</td>
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<td>Paramecium bursaria</td>
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<td>Cylindrid elongata</td>
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other container habitats.

Description of field sites

I have used the pitcher plant populations from three bogs. All three are within 6 miles of the University of Michigan Biological Station, Cheboygan County, Michigan: Mud Lake Bog (84° 35' 44" W., 45° 36' 26" N.), Livingston Bog (84° 38' 43" W., 45° 32' 29" N.) and Vestal Bog (84° 38' 49" W., 45° 31' 54" N.). Gates (1942) gives an account of all three bogs.

Vestal Bog has an extremely small population of pitcher plants, numbering less than eight individuals and 50 leaves. I studied this population because of the virtual absence of mosquito larvae in the pitchers, which permitted the study of protozoan community structure without any effects of predation. Despite the fact that Livingston Bog is only about 400 m from Vestal Bog, there was only one larva of Wyeomyia smithii in the Vestal Bog population during the entire summer of 1971.

Sampling techniques

The experiments, manipulations, and observations required that the populations of the mosquito larvae be enumerated and that the protozoan community be sampled. The enumeration of the mosquito larvae was done by removing the entire contents of a leaf using a plastic "turkey baster." The larvae were taken from the field to the laboratory for counting and were returned when necessary to the leaves from which they came before the next morning. The maximum volume of the leaves was measured at the time of the removal of the larvae by adding water to the empty leaves using an automatic pipette. Either this water was left in the leaf for leaves to be used in later experiments, or the water was replaced by the original pitcher fluid and detritus if long-term observations were being made.

The sampling of the protozoan communities utilized 15 cm Pasteur micropipettes with 2 ml rubber bulbs. Mixing of the fluid in the leaf before sampling was accomplished through the emptying and filling of the pipette and through the ejection of air at the bottom of the pitcher. A sample of about 1.5 ml was then collected and placed in an autoclaved screw-cap vial and returned to the laboratory.

In the laboratory 0.5 ml of pitcher fluid was taken from the vial with a pipette and placed on a depression spot plate. While this fluid was examined under the dissecting microscope (magnifications 15–45 times), the larger ciliates and rotifers were identified and enumerated. In order to identify and count the smaller forms, a 0.05 ml sample was taken from the depression slide with a pipette. This sample was placed upon a glass slide and covered with a No. 00 coverslip. The entire field was scanned at 78X under bright field illumination, and all taxa encountered were identified using Kahl (1930) and Kudo (1946); generally this could be done at 78X but frequent use was made of the higher magnifications (125X, 200X, 312X, and 500X) where phase contrast illumination was used. During the initial scanning, counts were made for species in the low-abundance range. For taxa not tallied during the initial scan and for species of greater abundance, further scans of the entire field were necessary. If the abundance of a taxon on the slide was high, then subsampling of the slide was necessary.

Measures of community structure

The protozoan samples are counts of the individuals of each tad and are extrapolated, where necessary, to number per 0.5 ml. From these data the total number of taxa (S) and the total number of individuals (N) are computed, and a measure of the evenness of distribution of individuals among taxa (H/\(H_{\text{max}}\)) is obtained (Pielou 1966). I prefer to examine the two components of diversity that are usually combined into a single measure of diversity; by using S and evenness separately I can determine how the community structure is changing without making arbitrary assumptions as to how each should be weighted in a single measure of diversity.

Description of experiments

Wyeomyia density observations

The purpose of these observations was to determine what densities of mosquito larvae could be encountered in the field at any one time and how these densities change through time. Density assessments were made at four times. The first sample of 273 leaves from Mud Lake Bog and Livingston Bog was taken between 30 April 1971 and 27 May 1971. This sampling was entirely from leaves produced during the previous year, and the sampling was done after the spring thaw but before any larvae were observed to have pupated. The second sample was also made on leaves produced during the previous year. In this case 172 leaves from both bogs were assessed during the period from 22 June 1971 to 4 July 1971. This period encompassed the main emergence for the overwintering brood as well as the development of the initial brood of the year.

The third sample was taken entirely from new leaves of the year at Mud Lake Bog, with a sample size of 193. The period of sampling was 10–27 July 1971, basically the time of development of the first brood of larvae in the new leaves. Finally a fall
sample was taken at Mud Lake Bog from 22 September to 12 October 1971 consisting of 128 leaves. All leaves sampled were produced during the year, and the larval population sampled would overwinter in the leaves. Sampling was done on leaves generally within the size 15–50 ml.

Field observations

To determine in general terms the relationship between the densities of predators and the structure of the protozoan community, a set of observations providing background information for the experimental field studies was made on both simultaneously in the field. The observations at Mud Lake Bog consisted of samples from 40 leaves. These leaves were sampled twice, 21–27 May 1971 and 25–26 June 1971. A similar set of observations was made at Livingston Bog on a sample of 32 leaves, 17–19 May 1971 and on 22 June 1971. These two sets of observations are pooled in the analysis as their comparative results were homogeneous.

A final set of field observations was carried out on a sample of 60 leaves at Mud Lake Bog on 22–27 September 1971.

Field experiments—May

From preliminary work I expected that experiments would be necessary to detect the specific patterns of community response. Three experiments were carried out, all at Mud Lake Bog, in May, August and October. The design of the August and October experiments depended heavily on the results of the May experiment.

The initial structure of the protozoan community is controlled in this and later experiments, and this structure influences the expected results and their interpretation. For the May experiment the initial protozoan inoculation consisted of a mixture of all of the protozoans contained in the fluid that had been taken from all of the leaves that were to be used for the experiment. This fluid contained about 12 taxa, about four more than would be expected in a leaf in which the protozoan community was at equilibrium in the absence of predation (Fig. 2a).

The May experiment was set up on a 6 x 4 analysis of variance design. The first factor consisted of six levels of density of third instar larvae: 0.0, 0.1, 0.2, 0.4, 0.8, and 1.6 larvae per ml. The second factor was size of leaf, which served as blocks in the experimental design. The leaf sizes were grouped around 20, 25, 30, and 35 ml. Each combination of predator density and leaf size was then replicated 6 times for a total of 144 replications. During the course of the experiment one leaf was damaged and lost to the experiment.

Between 30 April and 7 May, 197 leaves were chosen in the field for use in the experiment. Those in the appropriate size range were assigned to treatment levels at random. The experiment was put into the field on 10–13 May 1971. Sampling of part or all of the replicates was carried out on three dates to determine the appropriate time for the sampling of the entire experiment. On 25 and 26 May 1971 the first 2 replicates (48 leaves total) were sampled. On 31 May and 1 June 1971 these same leaves were sampled, and by a quick comparison of the results
for number of taxa only, it was decided to sample the remaining four replicates. On 10 June 1971 the first replicate was sampled again. Analysis of co-variance run at a later time showed that the responses of the protozoan community were homogeneous at these three periods.

Field experiments—October

This experiment was identical to the May experiment. The same treatment levels, block sizes, and number of replications were used. Third instar larvae were used. The experiment was put in the field on 4 and 5 October 1971 and was sampled between 22 and 31 October 1971. The purpose of the experiment was to confirm the results obtained from the May experiment.

Field experiments—August

Two basic experimental conditions were changed from the May experiment, the absolute predation intensities and the initial condition of the protozoan communities. After the May experiment, I decided to add a second initial condition for the initial protozoan community, consisting of a low number of species in contrast to the high number of species used as the other initial condition. In the low initial condition, cultures of three protozoans Colpidium campyllum, Bodo sp., and Polytomella agilis, were obtained at high densities.

The design of this experiment was as follows: there were six levels of predator densities, 0.0, 0.05, 0.1, 0.2, 0.4, and 0.8 larvae per ml; two initial protozoa community types, low S (3 taxa) and high S (about 12 taxa). For the low-S condition there were six leaf sizes used: 20, 25, 30, 40, 50, and 60 ml with two replications at each of the three factors for a total of 72 replications. For the high-S condition there were five leaf sizes, 20, 25, 30, 50, and 60 ml with two replications at each for a total of 60 replications.

The experiment was placed in the field between 30 July and 5 August 1971 and was sampled on 16-20 August 1971. The pitchers were covered with caps made of 16 x 20 mesh plastic window screen to prevent the access of female Wyeomyia smithii to the leaves.

For each of the experiments described above, detritus beyond that already contained in the pitcher fluid was added to each leaf at the beginning of the

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Fig. 3. Frequency distribution of the number of larvae of Wyeomyia per leaf for (a) May (N = 273), (b) June (N = 172), (c) July (N = 193), and (d) September (N = 128).
experiment—meal worms, flies or grasshoppers, all dead at the time of addition.

**Vestal Bog observations**

Because of the lack of mosquito larvae in the pitcher plants in the population at Vestal Bog, a series of observations was made on the protozoan communities that developed in the absence of predation. Late June and early July were very dry in northern Michigan during 1971, so that few observations could be made on these communities until about 25 July, even though many leaves were already open by that time. A total of 35 leaves were eventually sampled but not all of these were available at every time period, as some did not open until August and some were damaged or went dry for unexplained reasons. For 23 of the leaves, very good estimates of leaf age were obtained; for the remaining 12, relative ages were available, but with less accuracy. During the period of sampling, from 6 July to 22 October 1971, 209 samples were taken from the 35 leaves, 11 samples being the most from any one leaf.

**RESULTS**

**Larval density**

A particularly significant aspect of the pitcher plant system is that the larvae of *Wyeomyia smithii* can be found at a variety of densities in different leaves in the same bog. Since this permits study of the effect of different predator densities, the distribution of densities was quantified four times of the year. Fig. 3 illustrates the patterns that were observed during May, June, July, and September–October for the number of larvae per leaf. Table 2 gives the means, variances, and medians for both the number of larvae per leaf and the number of larvae per milliliter. The patterns for the number of larvae per leaf at the different times were compared, the data in eight groups: 0, 1–5, 6–10, 11–15, 16–20, 21–25, 26–35, and more than 35 larvae per leaf. The pattern for June (Fig. 3b) results from the relatively synchronous emergence of the overwintering brood without any significant input of new larvae from the newly emerged adults, which thus accounts for shifts to lower densities. The high median density of larvae per milliliter during July is the result of low rainfall during the period when the new pitchers of 1971 were opening, while the number of larvae per leaf was within normal values. Thus, at all times of the year there is significant variability in the densities of the mosquito larvae. Generally at least 10% of the leaves have densities less than 0.20 larvae per ml.

**Natural larval density and protozoan community structure**

On the basis of the results from a preliminary experiment and the observations of the densities of the larvae of *Wyeomyia smithii*, I expected to find significant regressions for the parameters of protozoan-community structure on larval density when the protozoans and mosquito larvae are sampled simultaneously from the field. The observations made during May, June, and September tend to confirm the expectation. The observed scatter plots along with the fitted regressions are presented in Fig. 4, 5, and 6, respectively. For each period the results for the total number of taxa (S), the common log of the total number of individuals (log(N + 1)), and evenness are presented. The results for May and June are similar to each other with respect to the direction and magnitudes of the responses of the protozoans to the different densities of mosquito larvae. As should be expected there is a significant negative slope for log(N + 1). The effect of the
density of mosquito larvae upon $S$ also gives a significant negative slope for both periods. In neither May nor June was there a detectable effect of larval densities upon evenness in the protozoan communities. The observations made during September show that there is no significant effect by the mosquito larvae upon any of the measures of community structure. The ineffectiveness of the larvae during the fall is attributable to the entrance of the third instar larvae into diapause; that is, larval density is no longer a good measure of predation intensity. Because of the large degree of variability caused both by sampling error and real differences between leaves, these observations are relatively insensitive for testing the original hypothesis. Detection of an initial rise in species number would be particularly
Fig. 6. Field observations during September of the relationships between the number of larvae of Wyeomyia per ml and (a) S \( (N = 60, \ Y = 2.50 - 0.32 X, \ r = -0.23, \ NS) \); (b) \( \log(N + 1) \) \( (N = 60, \ Y = 2.03 - 0.16 X, \ r = -0.13, \ NS) \) and (c) evenness \( (N = 35, \ Y = 0.51 - 0.06 X, \ r = -0.19, \ NS) \).

Field experiments

Field experimentation provides a more appropriate mechanism for testing the predation-diversity hypothesis because the response of the protozoan community may be compared among replicated groups at known and constant density levels, and some of the additional sources of variability such as nutrient availability can be held constant over all predation levels. The May and October experiments are analyzed by linear regression and the August experiment by covariance. The means and 95% confidence intervals for the means at each density level are presented for the May, August, and October experiments in Fig. 7, 8, and 9, respectively.

In the May experiment I used the high-S initial
condition, so that more species were originally present than would be expected in the absence of predation at equilibrium. The purpose of this experiment was to determine whether predation by the larvae of *Wyeomyia smithii* at intermediate densities could allow more taxa to remain in the community than at either higher or lower densities. Under these initial conditions if such a result were to occur, the following processes would most likely lead to the following result: during the time available (3 wk) competition among the protozoans in the absence of predation must act to eliminate some of the 12 taxa from the community; at the same time, where predation is moderate, the effect of predation in lowering prey densities must be to decrease competitive interactions and thereby allow more of the 12 taxa to remain in the system; at the very high densities predation could override the system and cause the direct elimination of taxa.

The results obtained from the May experiment are clear and striking. Increased density of *Wyeomyia smithii* causes a monotonically decrease in S (Fig. 7a). A comparison of the mean number of species at the intermediate density level with the number found where there were no mosquito larvae disproves the hypothesis that an increased number of protozoan species occurs in the presence of the mosquito larvae. The effect upon log(N + 1) (Fig. 7b) is significant and negatively monotonic, as would be expected. The effect upon evenness is qualitatively different from the above two and deserves more detailed consideration. The specific question with respect to evenness is whether or not predation can increase the evenness of distribution of individuals among the species. When predation results in the simplification of the community to one or no taxa, as occurs in 10 of the 143 leaves of this experiment, the question becomes meaningless and the measure undefined. These 10 cases have therefore been excluded from the analysis. The results from the remaining leaves show that there is a significant positive regression (Fig. 7c; Table 3) between evenness and the density levels, a result consistent with the original hypothesis. Although the results for H itself are not presented graphically, it is worth noting how H responds, since H may be thought of as being sensitive to both S and evenness. The effect of predation upon evenness is weaker than upon S, and therefore H shows

(b) S for the high-S initial condition (N = 60), (c) log(N + 1) (N = 132, Y = 3.48 - 0.82 X; r = −0.36, P < 0.0001), and (d) evenness (N = 132, Y = 0.46 + 0.20 X, r = 0.30, P < 0.001). Analysis for S for the combined low-S and high-S initial conditions above yields b = −0.99, and r = −0.16. (Also see Table 3.)
a significant negative regression with predation \( b = -0.28; F_{1,141} = 16.45 \). Thus, under conditions of high initial species numbers and when using third and fourth instar larvae, the system responded with negative monotonic effects upon \( S \) and \( \log(N + 1) \), and with positive effects upon evenness.

The August experiment was designed after study of the results from the May experiment. Two major factors were altered. First, the density levels of the six treatments were halved, and first and second instar larvae were used instead of third instar larvae. Second, I felt that the initial structure of the protozoan community might affect the results, so I established two initial conditions for the protozoan community, high \( S \) and low \( S \). A result consistent with the hypothesis for the high-\( S \) initial condition should occur in the manner described for the May experiment. For the low-\( S \) initial condition the processes would be somewhat different. The low-\( S \) initial condition consisted of the addition of three species, each at a very high density. Were the hypothesis valid under these conditions, colonization of the leaves by species not previously in the community would have to occur more quickly in the presence of predation than in its absence.

The results from the August experiment (Fig. 8; Table 3) show that there is again the expected negative effect of predation upon \( \log(N + 1) \), with the magnitude of the slope being less than that during the May experiment. There was no difference of slope between the low-\( S \) and high-\( S \) initial conditions. The significance of the regression for the number of taxa for the pooled high-\( S \) and low-\( S \) initial conditions is marginal, the probability being 0.052. The important fact is that the effect of predation upon the number of taxa is weaker under these conditions of lower predation intensity. The low-\( S \) and high-\( S \) initial conditions show homogeneous slopes for the effect of predation upon \( S \), but there is the expected difference between the adjusted means for the two groups (Fig. 8a, b). The effect of predation upon evenness (Fig. 8d) for this experiment is very similar to that observed for the May experiment, in both the magnitude of the regression coefficient and the significance of the regression; and the two initial conditions respond homogeneously. Under these conditions the effect of the evenness component on \( H \) is strong enough to cause a significant positive regression for \( H \) on predator density \( b = 0.26; F_{1,129} = 5.77 \). Thus, except for the change in the response of \( H \), there was little qualitative change between the May and August experiments, particularly with respect to the rejection of the hypothesis that predation will allow increased species numbers in the community.

The October experiment, identical in design to the May experiment, gave results different from the May and August experiments. These results confirm the field observations made at the same period of time: mosquito larvae have no effect upon protozoan community structure after the last part of September (Fig. 9). This is true even for \( \log(N + 1) \) which is otherwise the most sensitive indicator of the effects of predation.

**Vestal Bog observations**

The observations at Vestal Bog provide comparative information because of the almost total lack of *Wyeomyia smithii* in this population of pitcher plants, which permitted long-term observations under conditions of no predation. The patterns of development in the communities at Vestal Bog for \( S \), \( \log(N + 1) \) and evenness as functions of the ages of the leaves are presented in Fig. 2.

The results from Vestal Bog do provide further evidence on the nature of the influence of predation upon the protozoan communities. For one, the final values of the parameters of community structure are remarkably similar to the same parameters in the absence of predation during the May and August experiments. At Vestal Bog the average number of taxa is about 7.7, \( \log(N + 1) \) is about 3.55, and evenness is about 0.56. These compare with the intercept values for the May experiment of 8.8, 3.49, and 0.46, respectively. Second, comparison of the Vestal Bog data with the observational data from Mud Lake Bog and Livingston Bog at all times of the year clearly shows the effect of mosquito larvae in lowering the number of taxa.
The patterns of the development of community structure with time are interesting to note (Fig. 2). The curve for the number of taxa clearly represents a monotonically increasing function rather than one showing some type of oscillatory behavior. This pattern is consistent with the colonization data from other container-habitat protozoan communities (Maguire 1963a, 1971). This observation in itself may provide one indication why no increase in species numbers would be observed with predation in this system.

The time series curve for $S$ for all leaves (Fig. 2a) together is reasonably consistent with the curves for individual leaves, as there are very few examples of leaves in which there was any significant amount of oscillatory behavior. Only two of 17 leaves providing adequate data showed decreases of more than three species from a peak value. Representative curves from the individual leaves are shown in Fig. 10. More commonly (particularly for those leaves that opened in July) the equilibrium number of species appears to have been reached between 20 and 30 days after the initial examination of the leaf, with only minor variation from that point on. Fig. 10 also illustrates that much of the variability observed in Fig. 2a is due to differences between leaves.

The average pattern of development of evenness with time (Fig. 2c) is similar to that for $S$, but the variability is large. The pattern for log($N+1$) shows that there is a fast rise to a maximum value with very little variation after that time.

**Evidence for competition**

A sound operational definition for the detection of competition in the community in the absence of predation can be based upon the following reasoning. In a community where the competitive interactions are very weak or entirely absent, predation at various increasing intensities should result in the steady decrease of both the density and frequency of occurrence of all species in the community (Fig. 11a). The speed of elimination of individual species from the community will be dependent upon the rates of increase of the prey species and the selectivity of the predator, but the overall pattern of steady decreases for all species should not be dependent upon either. At the other extreme, where competitive interactions
among the species of the community are strong, another pattern of response could be observed (Fig. 11b) that will depend primarily upon the selectivity of the predator, and to a lesser degree upon the rates of increase of the species. If species are rare or absent from the community because of competition, and if predation falls most heavily upon the common species, then the pattern of Fig. 11b will be observed. However, this pattern would not be observed if the predation fell most heavily upon the rarer species. Under some conditions nonselective predation could lead to this pattern as well. Thus, a conservative definition for the existence of competition in the community should be the observation of patterns of species responses such as those in Fig. 11b.

The definition is conservative because many competitive situations will not be detected in this manner and it requires that the community be subjected to different intensities of predation through some natural or experimental procedure. It should also be pointed out that a system need not show increased species numbers in order to show the pattern in Fig. 11b. The definition would best be applied to data on the number of individuals per species, but can also be applied to relative frequency of occurrence in replicated systems.

This operational definition was applied to the species data obtained from the May experiment using the information on frequency of occurrence for 10 taxa (Fig. 12). Three patterns can be seen clearly in their responses. First, a group homogeneous within itself is markedly affected by the increasing levels of predation (Fig. 12a). The remaining two groups have a more even frequency distribution than the first, there being less of an overall effect of predation upon the frequency of occurrence. The second group contains three species that are clearly very little affected by predation, whereas the third
TABLE 4. The number of significant correlations at each predator level for one leaf from Vestal Bog; the significance level is 0.05

<table>
<thead>
<tr>
<th>Predator levels</th>
<th>0.0</th>
<th>0.1</th>
<th>0.2</th>
<th>0.4</th>
<th>0.8</th>
<th>1.6</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total number of coefficients calculated</td>
<td>171</td>
<td>136</td>
<td>120</td>
<td>105</td>
<td>105</td>
<td>120</td>
</tr>
<tr>
<td>Total number of significant correlations</td>
<td>13</td>
<td>25</td>
<td>19</td>
<td>41</td>
<td>16</td>
<td>37</td>
</tr>
<tr>
<td>Total number of significant negative correlations</td>
<td>7</td>
<td>2</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

Another indirect method for assessing the importance of competitive interactions among species is to examine the pairwise correlations between the number of individuals of the species in the community. This was done for the data from the May experiment, where the raw data were log transformed before the correlations were computed. Correlations between every species pair were computed individually at each level of predator density. At every level of predation there were many more significant correlations than would be expected by chance alone. With no predation 7 of 13 significant correlations ($P < 0.05$) were negative. In the presence of predation almost all significant correlations were positive, no significant negative correlations occurring above 0.1 larvae per ml (Table 4). None of the significant negative correlations involved species which increased in frequency of occurrence with predation.

The data from Vestal Bog provide another means of assessing the importance of competition in this system. If independence among species in the protozoan community is assumed, each species would be expected to increase in frequency and density as a monotonic function of time, and to reach some maximum as a function of its colonization rate and the suitability of the pitcher plant habitat. An interactive model, on the other hand, would predict that some species would increase in density and frequency during the initial phases of community development, only to be eliminated at some later point in time because of the increasing intensity of competition from one or more species. The observations made at Vestal Bog display a pattern that is consistent with the interactive model (Fig. 13). Of the 15 taxa that occurred with some regularity at some time during these observations, four appear to follow a pattern of initial increase followed by decreases (Fig. 13b): Colpoda inflata, C. cucullus, C. steini and Platyophrya spumacola. The other group generally shows comparatively smooth increases in relative frequency with age of the leaf. What makes these results even
more striking is that during the time when the four species are showing their declines, the species curve (Fig. 2a) is continuing to increase. One possible explanation for these results is that the decline or elimination of the four species is caused by the interactions from the increasing numbers of other species.

An alternative interpretation could be that the results simply represent the responses of these particular species to unfavorable changes in the abiotic environment caused by the colder weather of the fall or to the aging of the leaves. This interpretation appears unfounded, however, as at least two of these species show increases in density and frequency of occurrence when larvae are removed from leaves of the same age during the fall at Mud Lake Bog. This would not be expected if environmental factors were the cause of the Vestal Bog observations. Furthermore these conclusions for the species of *Colpoda* agree with the conclusions of both Maguire (1963b) and Bamforth (1971) upon the role of competition in the distribution of species of *Colpoda*.

Thus, three sets of observations are consistent with the conclusion that some competitive interactions do exist among the species in the protozoan community. However, clearly whatever the magnitude of these competitive interactions may be, they are important for only a small subset of species, and that therefore competition is probably not an important structuring process in the absence of predation.

Some questions may be raised about whether the effect of *W. smithii* upon the protozoan community is primarily through predation or competition, since mosquito larvae are potentially capable of grazing upon the food resources of the protozoans (Bates 1949). I have two sets of data relating to this question. First, in a laboratory experiment where protozoan communities from the field were subjected to different densities of mosquito larvae, it was shown that the number of species was reduced in just 2 days to about four from about 10 when only two larvae were present in 20 ml of fluid. The time available for this reduction is inconsistent with the hypothesis that the species losses were caused by competition from the mosquito larvae. Second, the changes in density of the individual species in response to the different densities of mosquito larvae in the May and August experiments are consistent only with hypothesis of predation. The largest ciliates and the rotifers were affected very little, as were the smallest ciliates and flagellates. However, the densities of the moderate-sized ciliates and the larger flagellates were most strongly affected. These results could be due either to size selective predation by the mosquito larvae, or to nonselective predation with sufficient resources still available to the smaller species to allow them to maintain a high rate of division. Thus, although there may be some competition between the mosquito larvae and the protozoan community, clearly the major effect of the mosquito larvae is predation.

## Discussion

Given the different larval populations used during the three experiments, the results are consistent among themselves as well as with the descriptive studies. Except when the larvae of *Wyeomyia smithii* have entered diapause, the negative effect upon total number of individuals is strong. The negative effect upon *S* is strong with the larger instars, marginal with the younger instars, and absent during the period of larval diapause.

Despite the attempts to create a variety of initial conditions enhancing the likelihood of observing more species in the presence of predation, no evidence supports the hypothesis for species numbers. If predation were to enhance species numbers in this system, the effect would have to occur at very low larval densities, and this could only be achieved in leaf sizes and larval densities rarely encountered in the field. Moreover, the lower absolute predation intensities used during the August experiment also resulted in no increase of *S* above the value found in the absence of predation. From these results we must conclude that the pattern of response of *S* to predation in this system is of the negative monotonic type presented in general form in Fig. 1b. The probable causes for this result will be discussed below.

The effect of predation upon the evenness component of diversity is consistent with the original hypothesis and is observable except during larval diapause. Despite the ability to detect a significant positive effect, relatively little of the variability in evenness has been accounted for even under the more controlled experimental conditions. Hall et al. (1970) have discussed the difficulties of working with relative abundance data on systems capable of responding over relatively short time periods. In particular they point out that even if the response pattern from pond to pond (or in this case from leaf to leaf) were to follow the same pattern, the patterns are not likely to be synchronous with each other, thus analysis and detection of real differences would be difficult.

The effect of predation upon evenness in these experiments remains doubtful, however. The increased evenness might result simply from the error involved in sampling a less dense community where the relative abundances are otherwise unaltered. To test this possibility, data from a leaf from Vestal Bog was subjected to a rarefaction procedure which took into account the differential sampling efficiencies for large and small organisms. The values of *S*, *N*, and
evenness were all initially within the expected range for a leaf without predators. Rarefaction was done three times to decrease the total density in proportion to the decrease expected from the August experiment. The results showed that there was some expected increase in evenness, but that its magnitude was about half that expected from the August results.

Finding what actually causes the increase in evenness can be approached with alternative explanations, one applying quite well to this system. The first possible mechanism would be one where an interactive, equilibrium community is preyed upon by a predator that is highly selective upon the species that is the best competitor in the community. This appears to be the mechanism for the shifts in evenness observed by Hall et al. (1970). This is not a likely cause for the results in the pitcher plant system, however, for the following reasons: the community shows few negative interactions among the protozoan species, and from my own observations the mosquito larvae appear to be very generalized feeders. A second, more plausible, explanation for this system has been proposed by Hairston (1964). He argues that evenness values for communities controlled by predation will be greater than where the populations are resource limited. Under predator limitation the abundances of the individual species will be largely determined by their relative rates of increase, parameters that are highly sensitive to changes in environmental parameters. Under resource limitation the abundances will be determined by competitive relationships that should be much less sensitive to environmental perturbations. Thus, in the pitcher plant system it is postulated that at the moderate and high predator densities, the species abundances are controlled by the relative rates of increase that are subject to irregular fluctuations. This leads to higher evenness with predation than without it. This interpretation is supported by the sharp drop in the number of significant negative correlations between species as predation increased in the May experiment (see Table 4).

Now the probable causes for the observed response of species numbers to predation in this system may be considered. This can best be done within a framework of parameters that appear to be necessary and sufficient to explain how prey community structure will respond to the action of predators in general. My framework comes partly from the theory in the literature (Slobodkin 1961, Parrish and Sails 1970, Cramer and May 1972, Harper 1969, Janzen 1970, Maguire 1971, MacArthur 1972, Paine 1966, 1969a, 1971, Paine and Vadas 1969, Spight 1967) and partly from my own analysis of pertinent experiments and observations in the literature. Briefly, eight parameters are most important: (1) the intensity of predation; (2) the degree of permanent spatial structuring in the prey community; (3) the mechanism of predation (e.g., removal vs. cropping); (4) the patterning of predation intensity in time; (5) the importance of interspecific competition with respect to other factors in determining the structure of the prey community in the absence of predation; (6) the pattern of predation (e.g., selective, switching, generalized); (7) the relative distribution of competitive abilities among species in the prey community; (8) the correlation between competitive ability and intrinsic rate of increase.

Four sets of values for these parameters apparently should result in the observation of increased species numbers in the presence of predation. I will describe each of these briefly and then discuss why the pitcher plant system does not appear to fall within any of the four.

First, enhancement of species numbers through spatial heterogeneity may result from the ability of the predator functionally to create heterogeneity through its mode of predation (e.g., Paine 1966). That is, the predator clears a unit of the environment of all or most prey organisms and in time moves from one unit of space to another. In effect this implies that the spatial unit for the prey is much smaller than the spatial unit for the predator, so that the predator must search over a large area compared to the mobility of its prey. This mechanism also depends upon the mode of predation (i.e., prey must be removed) and the availability of propagules of species that normally would not compete well in the system. Such a mechanism for the enhancement of species numbers is not available in the pitcher plant system however. The appropriate spatial unit, the individual pitcher plant leaf, is the same size for both the mosquito larvae and their protozoan prey. Moreover, individual larvae are not free to move from leaf to leaf in response to their own removal of prey from the pitchers. And, it is not known whether the choice of leaves by the female for oviposition sites is determined by the availability of food or the presence of other larvae. Thus, because of the structure of this system and the life history of Wyeomyia smithii, the larvae do not appear capable of functionally creating heterogeneity within the pitcher plant system that would result in more species of protozoans being present in any one leaf at any one time or in the population as a whole.

Second, another disequilibrium explanation has been proposed by Hutchinson (1961), who argued that temporal heterogeneity of the physical environment is a possible mechanism for the maintenance of diversity in plankton communities. He concludes that if the time scales of the environmental fluctuations and the reproductive time constants of the
species are of the same order of magnitude, then the system in question may not ever reach equilibrium. Such a system, he argues, would have more species than one which does go to equilibrium (e.g., Cairns et al. 1971). Equilibrium would occur if the two time constants are different by orders of magnitude. Such an argument can be applied to the pitcher plant system, where fluctuations of the densities of the mosquito larvae replace environmental fluctuations. In this case, because the time constants for the protozoans and mosquito larvae are so disparate, hours vs. weeks, we would not expect that more species would occur with predation (and its fluctuations) than in the long-term absence of predation. This is because the protozoan communities are capable of reaching equilibrium within the time periods of density changes that are appropriate to the mosquito larvae. Thus, the two possible disequilibrium mechanisms for the enhancement of species numbers do not appear to be applicable for this system.

We may now consider the two sets of factors leading to the enhancement of species numbers under equilibrium between the predator and prey community. First, there may be systems where the predator is highly specialized or at least selective at any given time. In these systems if there is a highly skewed distribution of competitive abilities, a very few species being highly effective competitors, species numbers should increase with predation. Other distributions of competitive abilities would not yield the same result. Second, in systems where the predation is highly generalized, species numbers will increase only where there is a general negative relationship between competitive abilities and potential reproductive rates. However, both sets of factors depend upon the prey community being competitive in the absence of predation. This factor is crucial. A system that is noninteractive, either because of extreme environmental heterogeneity or very different resource requirements of prey species, is not likely to show enhancement of species numbers in the presence of predation. That is, the factors that determine species numbers in these essentially noninteractive communities are not affected by the action of the predator. In a competitive community predation may (but need not necessarily) influence the factors that determine species numbers; predation, by lowering prey densities in either of the manners described above, may free the resources for which competition previously occurred. The lack of observations of strong interactions among the protozoans in the pitcher plants is therefore quite significant. And if these observations were to be eventually supported by the appropriate experimental results, it would indicate that regardless of the pattern of predation, which in this case does appear to be quite generalized, and regardless of any possible correlations between rates of increase and competitive abilities, species numbers should not increase under predation in this system.

I have been able to differentiate unequivocally between two fundamental patterns (Fig. 1) of the effect of predation upon prey species numbers. This result points to the need for more detailed studies on the effect of predation upon prey community structure before generalizations about it can be made. Clearly in many systems predation may have a major effect on prey community structure, but the nature of the effect appears to differ between systems, some systems showing increases in both species numbers and evenness, some showing little if any effect upon species numbers but large effects upon evenness and species composition, and others (such as this study) showing increased evenness with decreased species numbers. It is possible to identify certain differences between systems that appear to cause these different response patterns and to make theoretical predictions as to how as yet unstudied systems should respond. Many of the critical experiments designed specifically to test the importance of the factors that may determine the nature of the interaction between the predator and its prey community remain to be done.

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