POPULATION OSCILLATIONS IN CONTINUOUS CULTURE OF THE PREY-PREDATOR SYSTEMS *ESCHERICHIA COLI* - *DICTYOSTELIUM DISCOIDEUM* AND *PROTEUS VULGARIS* - *DICTYOSTELIUM DISCOIDEUM*

by

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INTRODUCTION

Mixed cultures occur in most natural environments such as rumen, streams, lakes, soil, and the like. The most common mixed culture operations designed by man are those for biological waste treatment processes such as trickling filters, sludge digestors, activated sludge processes, and lagoons. Microorganisms in these units digest organic material and convert it to microbial growth and mineral matter. There are several possible types of microbial interaction which may occur. A list is given in Table 1. Many of these microbial interactions can occur simultaneously in biological waste treatment processes. Hence, the study of mixed cultures can lead to better understanding of waste treatment, fermentation processes, and the population dynamics of natural waters.

In this research a particular type of microbial association, predation was investigated with myxamoeba feeding upon bacteria. The object of this study was:

1. To characterize the relationship in terms of expected population oscillations occurring in a continuous flow culture of either Escherichia coli - Dictyostelium discoideum or Proteus vulgaris - Dictyostelium discoideum.
2. To investigate the effects of dilution rate on the population oscillations.

Continuous flow techniques and population enumerations with a Coulter Counter were employed for these investigations. Continuous flow of medium presents a more constant environment for microbial growth than do batch

methods. The Coulter Counter will determine and resolve populations very easy and quickly by size differences.

Table 1. Microbial Interactions

Type	Definition
Antibiosis	excretion of a factor harmful to the other
Competition	a race for nutrients and space
Commensalism	one lives off another without helping or harming it
Mutualism	each benefits the growth of the other
Predation	one feeds on another
Parasitism	one steals from another
Synergism	the combination synthesizes by cooperative metabolism

REVIEW OF LITERATURE

A. Theory of Mixed Cultures

Lotka (1925) presented mathematical equations dealing with the relations between interdependent species. These relations can take on a variety of forms. Most fundamental, perhaps, is that form of interdependence in which one species S_1 serves as food to another species S_2, so that in this sense S_1 becomes transformed into S_2, thus

$$S_1 \longrightarrow S_2$$

Four different types of this form of interdependence are possible.

(1) The organism S_2 kills S_1 outright in the process of feeding upon it. We might term S_2 in such a case an episite of S_1.

(2) The organism S_2 lives on S_1 without killing it outright, being parasitic upon S_1.

(3) The organism S_2 is saprophagous or saprophytic, feeding upon the cadavers of S_1 after death from other causes; or, S_2 may live on waste products discharged by S_1.

(4) The organisms S_1 and S_2 live in partnership which is in some degree mutually beneficial (symbiosis).

In general, the rate of growth $\frac{dX_i}{dt}$ of any one of these biological species S_i will be a function of the abundance X_i in which it and each of the other species is presented; this rate of growth will also be a function of the environment defined by the parameter P_i as well as the character of the biological species defined by the parameter Q_i. Therefore, the

fundamental equation was summarized as follows:

$$\frac{dX_i}{dt} = F_i\ (X_1,\ X_2 - - - - - X_n;\ P,\ Q) \qquad (1)$$

For the further discussion of equation (1) it is desirable to express these relations, not in terms of the masses X_i, but in terms of the excess x_i of each mass X_i over its corresponding equilibrium value C_i,

$$x_i = X_i - C_i$$

The equation (1) then takes the form

$$\frac{dX_i}{dt} = f_i\ (x_1,\ x_2 - - - - - x_n) \qquad (2)$$

The parameters P, Q were omitted for the sake of brevity. Expanding the right hand member by Taylor's theorem, we obtain

$$\frac{dx_1}{dt} = a_{11}x_1 + a_{12}x_2 + - - - \qquad (3)$$

$$\frac{dx_2}{dt} = a_{21}x_1 + a_{22}x_2 + - - - \qquad (4)$$

A general solution of the above equations will lead to

$$x_1 = G_{1,1}e^{\lambda,t} + G_{1,2}e^{\lambda_2 t} + - - - + G_{1,n}e^{\lambda_n t} \qquad (5)$$

$$x_2 = G_{2,1}e^{\lambda,t} + G_{2,2}\ e^{\lambda_2 t} + - - - + G_{2,n}e^{\lambda_n t} \qquad (6)$$

When X_i = mass of each organism

x_i = excess of each mass X_i over its corresponding equilibrium value

C_i = equilibrium value

a_{ij} = coefficients

G_i = constants

n = arbitrary constants

$\lambda_1, \lambda_2 - - - \lambda_n$ = the n roots of the equation for λ

As a general rule, the following observation regarding the coefficients a_i in the several types of interdependence enumerated above were made:

(1) S_2 lives on S_1 by killing S_1 outright (episitic type). In this case, S_1 is advantageous to the growth of S_2, so that

$$a_{12}<0 \qquad a_{21}>0$$

(2) S_2 parasitic upon S_1.

Here $a_{12}<0$, $a_{21}>0$, as in the episitic type.

(3) S_2 saprophytic upon S_1, or living upon waste products of S_1.

Here $a_{12} \geq 0$, $a_{21} \geq 0$

(4) S_2 feeds on S_1, but at the same time cultivates it in symbiosis.

Here $a_{12}>0$, $a_{21}>0$.

The characteristic equation for two variables is

$$\begin{vmatrix} a_{11}-\lambda & a_{12} \\ a_{21} & a_{22}-\lambda \end{vmatrix} = 0 \qquad (7)$$

or, in expanded form

$$\lambda^2 - (a_{11} + a_{22})\lambda + (a_{11}a_{22} - a_{21}a_{12}) = 0$$

$$\lambda = \tfrac{1}{2}\left[(a_{11} + a_{22}) \pm \sqrt{(a_{11} - a_{22})^2 + 4a_{21}a_{12}}\right]$$

Volterra (1927) stated three laws of fluctuation of two species, one preying on the other: 1. Populations of the two species may oscillate forever about the stationary state, 2. The asymptotic mean numbers of the species may be independent of initial state and equal to the stationary state values, 3. If individuals of the two species are destroyed in proportion to their numbers, the mean number of the eaten species (prey) increases while that of the preying species (predator) decreases. Equations were also presented to describe the fluctuation in numbers of a prey-predator system taking into account a lag between change in food supply and change in rate of increase of preying species.

Gause and Witt (1935) discussed the theory of the growth of mixed population of two species from the viewpoint of natural selection. In certain cases the result of two competing species is dependent upon the initial proportion of the two species. They concluded that the relative adaptability of two species is a rather variable feature.

Herbert, et al (1956) developed mathematical equations based on the Monod (1942) theory for the growth of bacteria in the continuous culture. The essential assumption is that the microbial growth occurs at a constant rate and in a constant environment (under steady-state conditions). These environmental factors include pH, concentrations of nutrients, metabolic products and oxygen supply.

In order to develop these mathematical equations, consider bacteria growing in a completely-mixed continuous culture apparatus. Sterile growth medium is fed into the culture vessel at a steady flow-rate and culture fluid emerges from it at the same rate, a constant-level or

similar device keeping the volume in the vessel constant. Complete mixing is assumed so that the entering growth medium is instantaneously and uniformly dispersed throughout the vessel, the inflowing medium has a single growth limiting organic substrate at a concentration S_R. It is assumed that all other substrates are present in excess, and the oxygen supply is always adequate. The variables within the control of the experimenter are the substrate concentration and flow rate of the incoming culture medium. Therefore, a complete theory has to explain how variations of these affect the growth rate, the concentrations of organisms and of substrate in the growth vessel.

When no organisms are being supplied from external sources, the growth rate in the continuous culture vessel is described by equation (1) and simultaneously the wash out rate is expressed by equation (2).

$$\frac{dx}{dt} = \mu x \text{ - - - - - (1)}$$

$$- \frac{dx}{dt} = Dx \text{ - - - - - (2)}$$

x = the concentration of organisms (dry weight of organisms/unit of volume at time t)

μ = specific growth rate

$= \frac{1}{x} \frac{dx}{dt}$ (the rate of increase/unit of organism concentration)

D = dilution rate

The net rate of increase of concentration of organisms is given by the simple balance equation (3):

$$\text{increase} = \text{growth} - \text{output}$$

$$\frac{dx}{dt} = \mu x - Dx \text{ - - - - - (3)}$$

Hence if $\mu > D$, $\frac{dx}{dt}$ is positive and the concentration of organisms will increase, while if $D > \mu$, $\frac{dx}{dt}$ is negative and the concentration of organism will decrease, eventually to zero; therefore the culture will be washed out of the culture vessel. Of course, μ changes with substrate concentration and is thus related to population. When $\mu = D$, $\frac{dx}{dt} = 0$ and x is constant; therefore we have a steady state in which the concentration of organisms does not change with time. Under such steady-state conditions, the specific growth rate, μ, of the organisms in the culture vessel is exactly equal to the dilution rate D. By itself the equation gives no information on what dilution rates make a steady state possible. To know this, we must also know how dilution rate affects the concentration of substrate in the culture vessel.

In the culture vessel, substrate is entering at a concentration S_r, being consumed by the organisms and flowing out at a concentration S. The net rate of change of substrate concentration is obtained by another balance equation (4)

$$\text{increase} = \text{input} - \text{output} - \text{consumption} \qquad (4)$$

Powell (1955) derived the relations between growth rate, generation time distribution and age distribution using continuous mixed cultures of Pseudomonas aeroginosa and B. megaterium and some assumptions.

B. Chemostat Apparatus

Novick and Szilard (1950) developed a device known as the "Chemostat" for keeping a bacterial population growing over an indefinite period of time. The contents of the vessel were stirred by bubbling air through it. Pressure was used instead of pumps.

C. Work Done with Mixed Cultures

Parker and Snyder (1961) used Streptococcus salivarius and Veillonella alcalescens for studying an interaction. The former provides lactic acid from carbohydrates; the latter utilizes lactate but not carbohydrate. Streptococcus salivarius and Veillonella alcalescens were grown in pure culture in individual chemostats and then combined for mixed growth in a second stage. The results indicated a distinct interaction between the strain employed in that the generation time for each species was markedly increased by the presence of the other.

Implicit in most theoretical treatments of growth kinetics of organisms in continuous culture was that mixed populations would not achieve a steady state - one population would over-grow and displace the others which were washed out. An exception was the situation wherein a mutant population existed in equilibrium with a parent population. In many natural environments which were essentially continuous culture systems, mixed populations were the rule, and steady-states were approached if not actually achieved. Contois and Yango (1964) studied steady-state microbial populations in continuous mixed culture. The

preliminary investigations were conducted in a conventional chemostat and in chemically defined media with the following mixed cultures:

(1) Bacterium/phage (*E. coli* and a coliphage):

After obtaining a steady state population for the bacterium (*Escherichia coli* ML30), phage was inoculated. The phage rose rapidly to a maximum and declined rapidly to a level which remained relatively constant in time. The apparent decrease in phage population was too rapid to be caused by wash-out and was presumably in part a result of multiple adsorption of phage by the host cells. The *E. coli* persisted at a population considerably below the initial level.

(2) Bacterium/Myxamoeba (*Aerobacter aerogenes* and *Dictyostelium discoideum*):

The bacterial population could grow independently while the myxamoeba population was entirely dependent on the bacteria as a source of food. The density of the bacteria was determined by their rate of growth, rate of wash out, and rate of engulfment by the myxamoeba. The population density of the myxamoeba was determined by their rate of growth and their rate of wash out.

(3) Bacterium/Yeast (*A. aerogenes* LMX and wild yeast):

The primary bacterial population grew independently of the yeast while the latter were dependent on the bacteria for one or more essential nutrients. At higher dilution rates the yeast were washed out of the culture.

(4) Bacterium/Bacterium:

Two strains of A. aerogenes were grown in mixed culture. One was Lac+, MR^+ and "acid sensitive", the other one was Lac^-, MR^-, and "acid resistant". Fluctuations in pH were observed and were highly correlated with total population density. The pH was the controlling factor. The primary population (Lac^+, MR^+, "acid sensitive") lowered the pH of the medium as a result of its growth, leading to its own inhibition and wash out. The lowered pH favored the secondary population (Lac^-, MR^-, "acid resistant") which grew and lead to an increased pH which favored the original primary population.

Bungay and Krieg (1965) reviewed some mixed culture aspects of dairy processes, commercial fermentations and biological waste treatment processes. The spontaneous mixed culture processes are those which arise naturally with little or no attempt to inoculate with specific organisms. Defined mixed cultures are intentionally inoculated with two or more characterized cultures. The goals of research on mixed cultures would seem to be understanding and prediction so that processes can be optimized.

Proper and Garver (1966) studied the mass culture of the protozoa Colpoda steinii; culture conditions, kinetics of growth, and yield were investigated for the ciliate grown with viable bacteria (E. coli) as the sole food source. At high bacterial concentrations (above 200 $mg/_l$)

the rate of growth of the protozoa is limited by the rate at which the bacteria are metabolized. As the bacterial concentration is decreased, growth of the protozoa is limited by the frequency with which they encounter bacteria.

A commensalism interaction between the yeast, Saccharomyces cerevisiae, and the bacterium, Proteus vulgaris, in countinuous culture was studied by Shindala, et al (1965). Niacin or near biochemical relatives was shown to be the essential factor supplied to the bacteria by the yeast, population estimates were made using a Coulter counter and were checked with Petroff-Hausser methods.

A mutualism interaction between Proteus vulgaris and Bacillus polymyxa in continuous culture was conducted by H. T. Yeoh (1967). P. vulagris needs a niacin-like factor and excretes biotin. B. polymyxa needs a biotin-like factor and excretes niacin. No steady-state was achieved in this interaction.

In recent work, Drake, et al (1966) presented experimental and theoretical considerations of the effect of the environment on the parameters of Volterra's equations. Volterra's theoretical equations could account for the effects by adding correcting terms for continuous culture. Dictyostelium discoideum and E. coli were used for this prey-predator investigation.

Raper (1935) isolated the species, Dictyostelium discoideum, from decaying leaves. This slime mold is a bacterial feeder and can be grown in association with a large variety of saprophytic bacteria.

METHODS AND MATERIALS

A. Description of Continuous Flow Apparatus

A typical continuous flow apparatus used in this study is shown in Figure 1. The culture vessel was a 500 ml. wide mouth Erlenmeyer flask. Air was filtered through a column of sterilized cotton and introduced into the flask with a hypodermic needle projecting through the rubber stopper. A magnetic stirring bar about one inch long was used to provide vigorous mixing. The culture was inoculated by pipetting through a 3/8 inch stainless steel entry pipe capped with a rubber stopper. The culture flask was partially immersed in a 25°C water bath.

The medium reservoir was either an 8-liter polypropylene container or 12-liter round bottom glass flask. A 1/4 inch glass tube projected through the rubber stopper to the bottom of the medium reservoir. Gum-rubber tubing connected from the reservoir to the culture vessel. The medium was fed with a Sigma-motor pump (Model T8, Middleport, New York) continuously at selected flow rates from the reservoir to the culture vessel. The different flow-rates were dependent upon the motor speed and the size of tubing. To maintain a constant volume in the culture vessel, a 3/16 inch glass over flow tube was drawn to a tip, and it was extended into the culture flask as shown in Figure 1. The entire apparatus was autoclaved at 15 psi for 20 minutes.

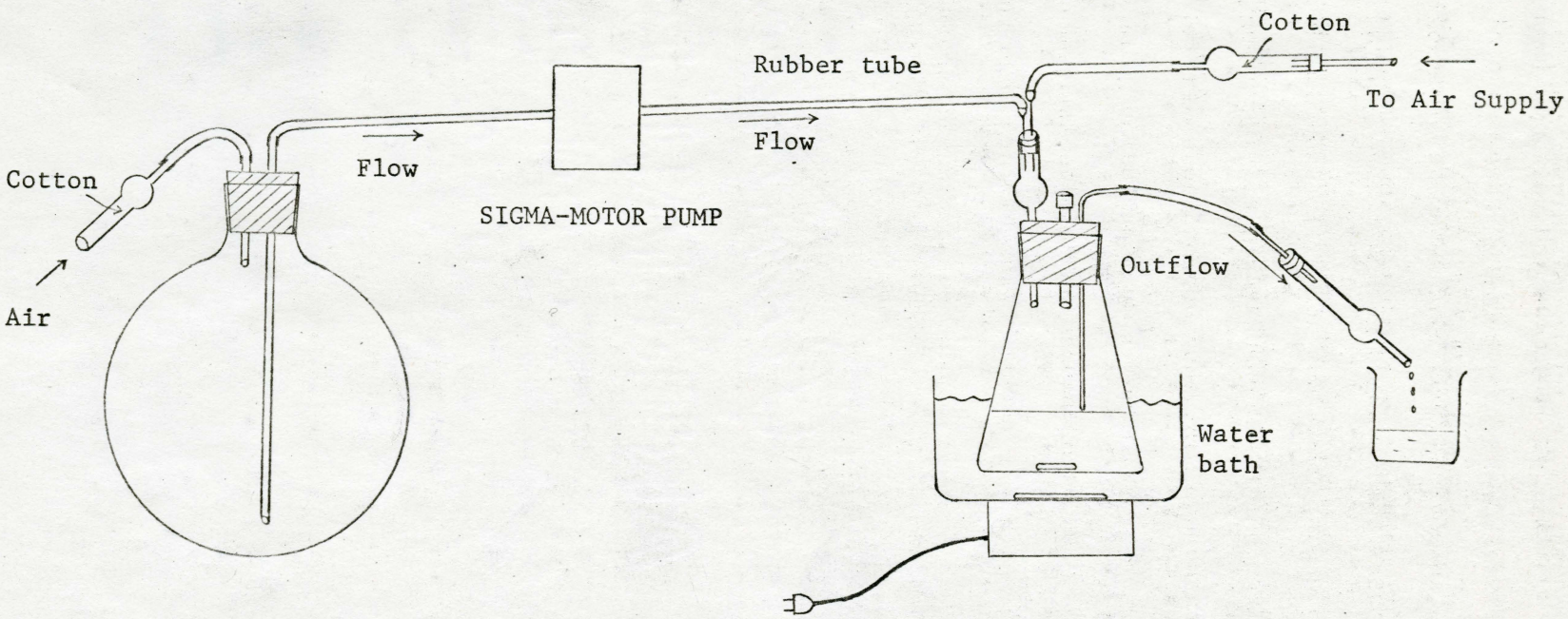

Figure 1. Continuous Culture Apparatus

B. Cultures and Their Maintenance

All bacterial cultures were obtained from the V.P.I. culture collection. Escherichia coli (ATCC # 9637) is a small rod, 0.5 by 1.0 to 3 microns, occurring singly, in pairs and in short chain, motile, gram negative, facultative, optimum temperature is 37°C. Proteus vulgaris is a small rod, 0.5 to 1.0 by 1.0 to 3.0 microns, occurring singly, in pairs, motile, gram negative, aerobic, optimum temperature is 37°C.

Dictyostelium discoideum was purchased from the General Biological Supply House, Chicago, Illinois. Dictyostelium discoideum is a cellular slime mold and a bacterial feeder exhibiting several stages in its life cycle; the first, as free-living myxamoeba; the second, as massed structures of cells exhibiting interdependence and coordination; and the third, as cells differentiating into several kinds of structures. The optimum growth temperature is 20° to 24°C.

For this study, stock cultures of E. coli and P. vulgaris were maintained on tryptic soy agar slants and stored at 10°C. Dictyostelium discoideum was subcultured on lactose agar slants containing 24 hour growths of E. coli and P. vulgaris. The slants were incubated at 25°C.

C. Composition of Media

Commercial lactose broth (Difco Laboratories, Detroit, Michigan) and tryptic soy broth (Fisher Scientific Company, Fair Lawn, N. J.) were used for the microbial interaction studies. The media concentration

was 0.2 gram of lactose broth per liter of distilled water for the E. coli and D. discoideum experiments, and 1 gram of tryptic soy broth per liter of distilled water for the P. vulgaris and D. discoideum experiments.

D. Operation of the Continuous Flow Apparatus

The culture vessel was charged by pumping about 250 ml. of nutrient broth and inoculated with 5 ml. of a 24 hours incubation of tryptic soy broth containing either E. coli or P. vulgaris. Growth in the culture vessel was then allowed to proceed batchwise for 14 hours at which time there was obvious turbidity. A loop needle was used to transfer several heads and stalks of D. discoideum from the lactose slant culture into a sterile tube of lactose broth or tryptic soy broth. This suspension was used to inoculate the culture vessel. Growth in the culture was then allowed to continue batchwise for 10 more hours to allow time for the D. discoideum to grow before continuous pumping was started. Gram stains were made every day to check for contamination. Samples of the culture were collected at regular time intervals and the population of each organism was determined with the Coulter Counter or by Petroff-Hausser counting.

Samples were taken from the effluent tube for Coulter Counter or Petroff-Hausser counting. One milliliter of the sample was pipetted into a 150 milliliter beaker which contained 99 milliliter of 0.9 percent membrane filtered saline to make a dilution of 1:100 for the Coulter procedure. A 50-micron orifice (Particle Data Laboratories, Elmhurst,

Illinois) was used throughout this study. Size distribution curves for E. coli and D. discoideum or P. vulgaris and D. discoideum in mixed cultures were prepared. The Coulter Counter settings for E. coli and D. discoideum, or P. vulgaris and D. discoideum in mixed cultures were obtained from the size distribution curves (Figure 1, 2) and listed in Table 2. Electronic counts were checked periodically with a Petroff-Hausser bacterial counter.

Table 2. Coulter Counter Settings for Counting E. coli, P. vulgaris and D. discoideum

Microorganism	Threshold	Aperture Current	Gain Index
E. coli	3 - 8	6	4
P. vulgaris	3 - 8	5	4
D. discoideum	8 - 13	6, 5	4

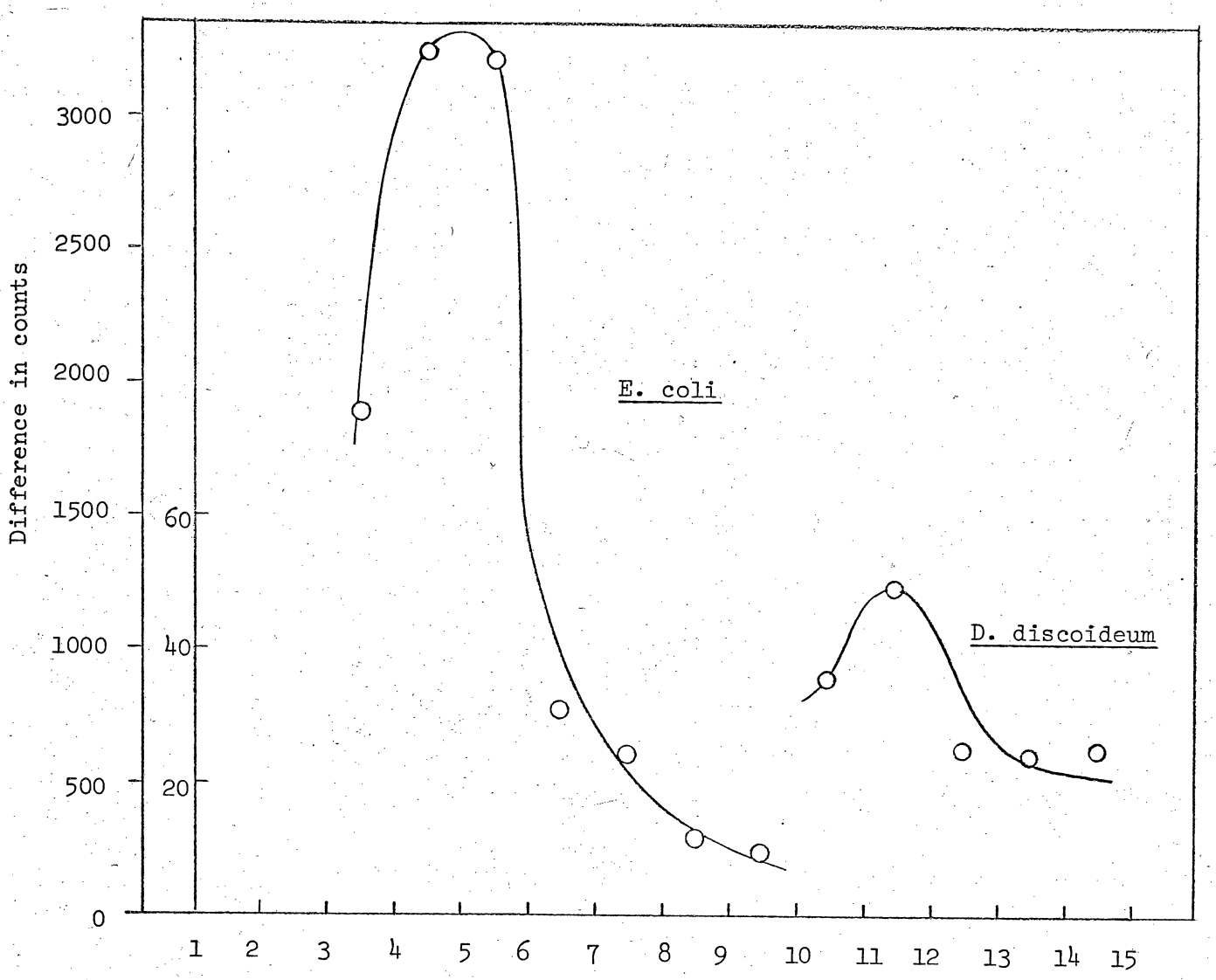

Figure 2. Size Distribution Curve for E. coli and D. discoideum

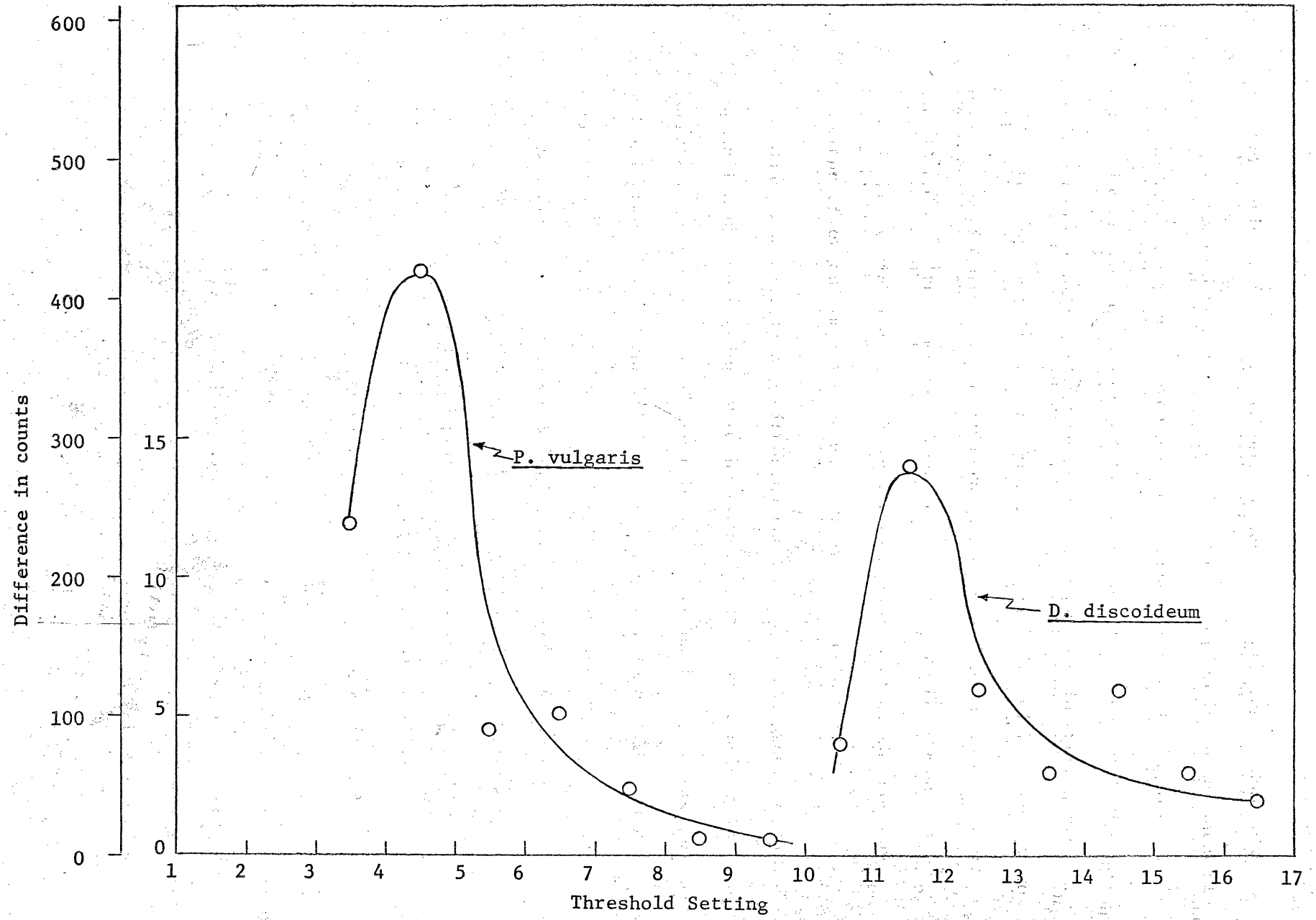

Figure 3. Size Distribution Curve for P. vulgaris and D. discoideum

RESULTS

A dilute medium was used on this experiment in order to obtain low bacterial populations which were desired to prevent the accumulation of toxic products which might effect the myxamoeba and to insure that aeration would not be a limiting factor.

Population levels of pure cultures of E. coli or P. vulgaris at various dilution rates were determined. Measurements were taken 10 to 12 hours after each new flow rate change to insure establishment of steady-state conditions. The population level gradually decreased as the dilution rate increased. The results are shown in Figure 4 and Figure 5.

Mixed growth experiments in continuous culture were performed with Escherichia coli and Dictyostelium discoideum, or Proteus vulgaris and Dictyostelium discoideum. Several runs were made and samples collected at selected time intervals for Coulter Counter counting.

Figures 6, 7 and 8 illustrate oscillatory populations for E. coli mixed with D. discoideum with 5 minutes sample intervals. The period of oscillation ranged from 25 to 35 minutes.

Figures 9 and 10 illustrate oscillatory populations for P. vulgaris mixed with D. discoideum with 10 minutes sample intervals. The period of oscillation ranged from 25 to 30 minutes.

Petroff-Hausser checks of Coulter Counter counts are represented in Table 3. The Petroff-Hausser methods generally give higher numbers of cells present because groups, doublets, clumps or flocs of E. coli and P. vulgaris give only single pulses on the Coulter Counter.

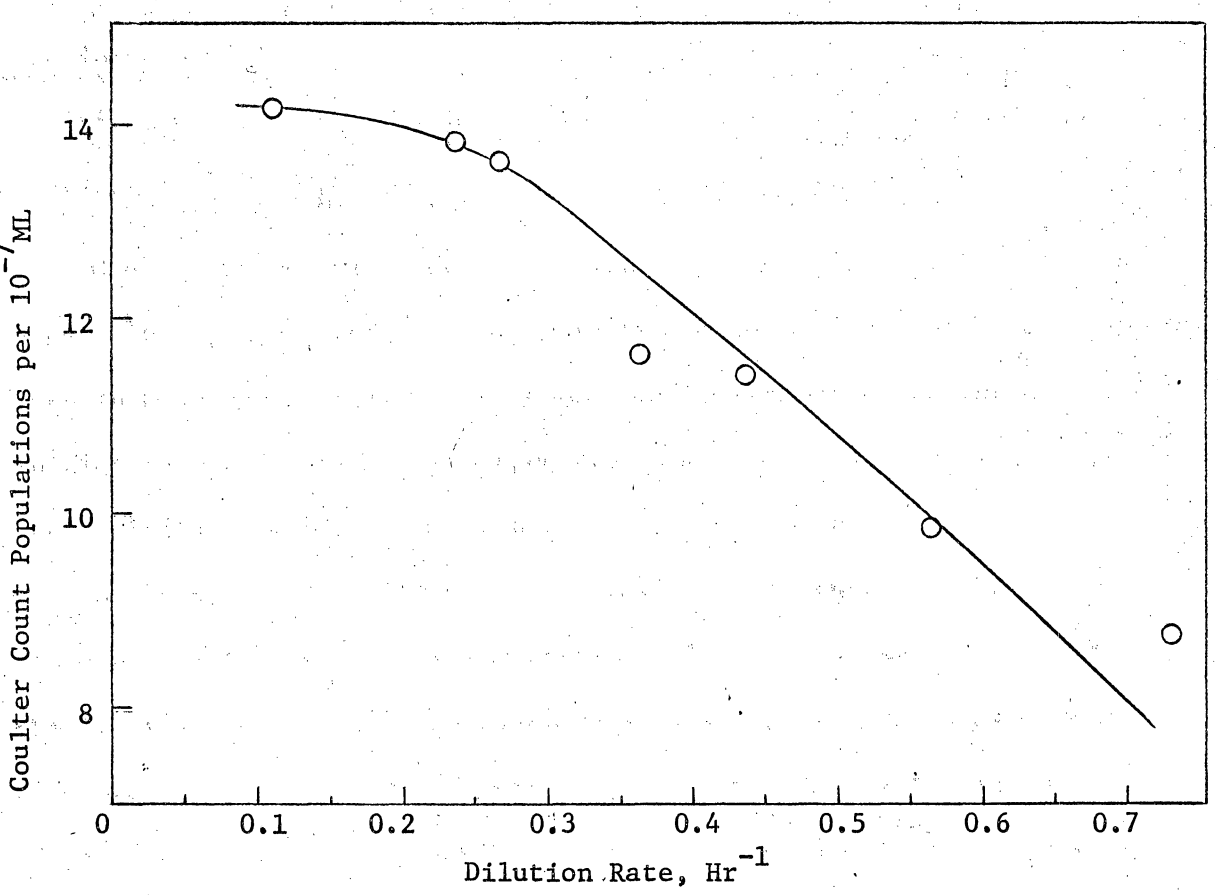

Figure 4. Population of E. coli at Different Dilution Rates.

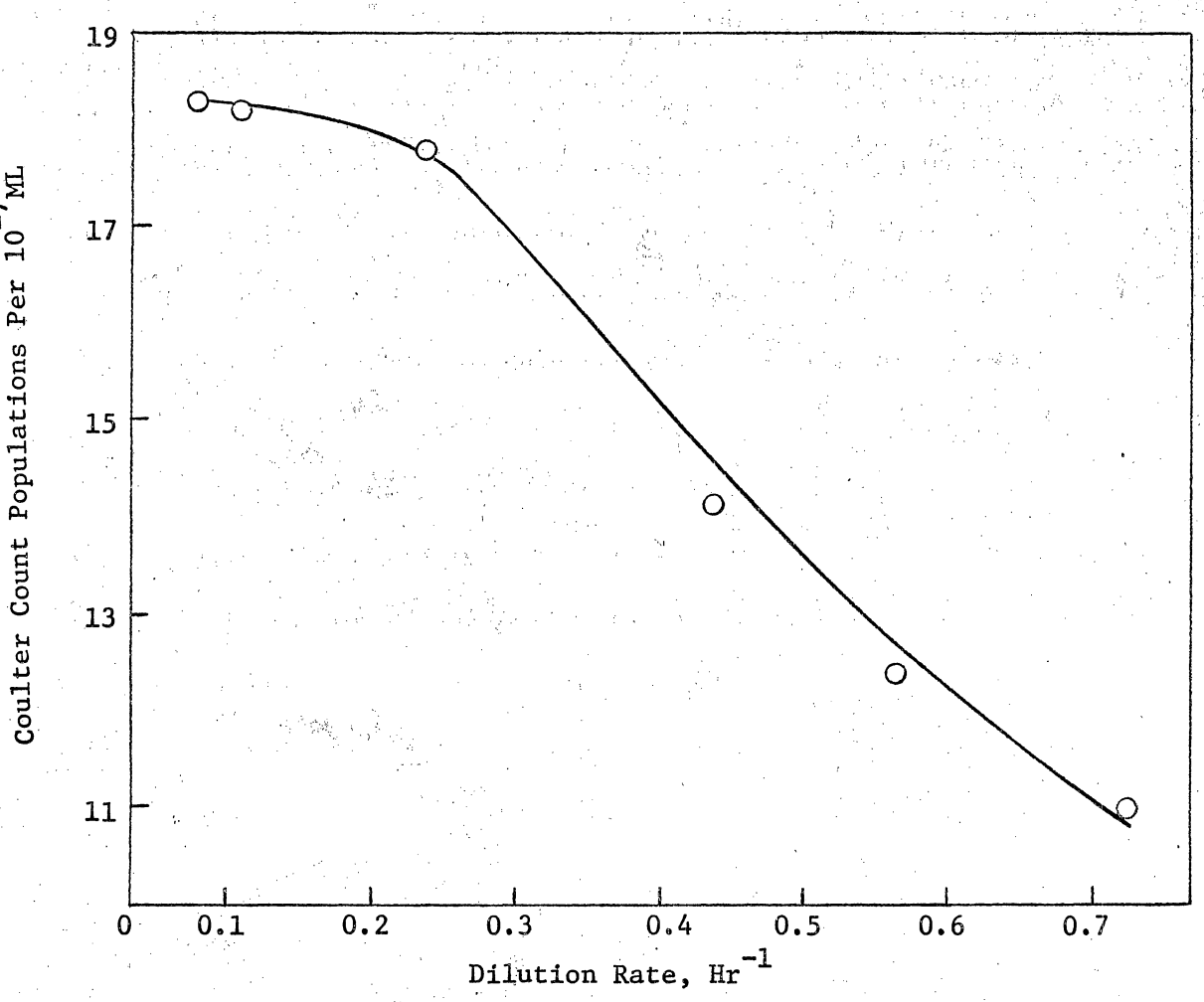

Figure 5. Population of P. vulgaris at Different Dilution Rates

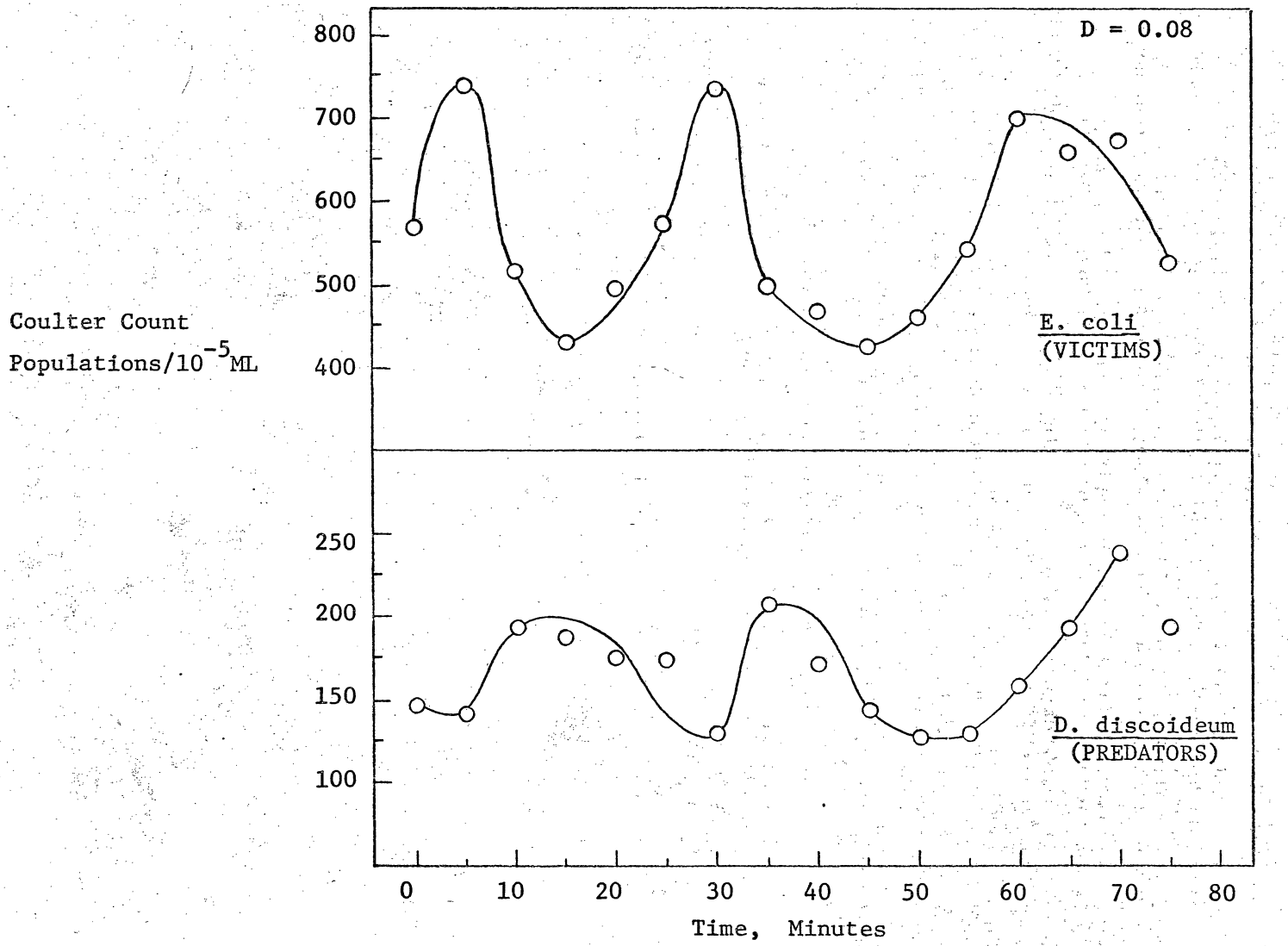

Figure 6. Fluctuation Populations in E. coli and D. discoideum Interaction

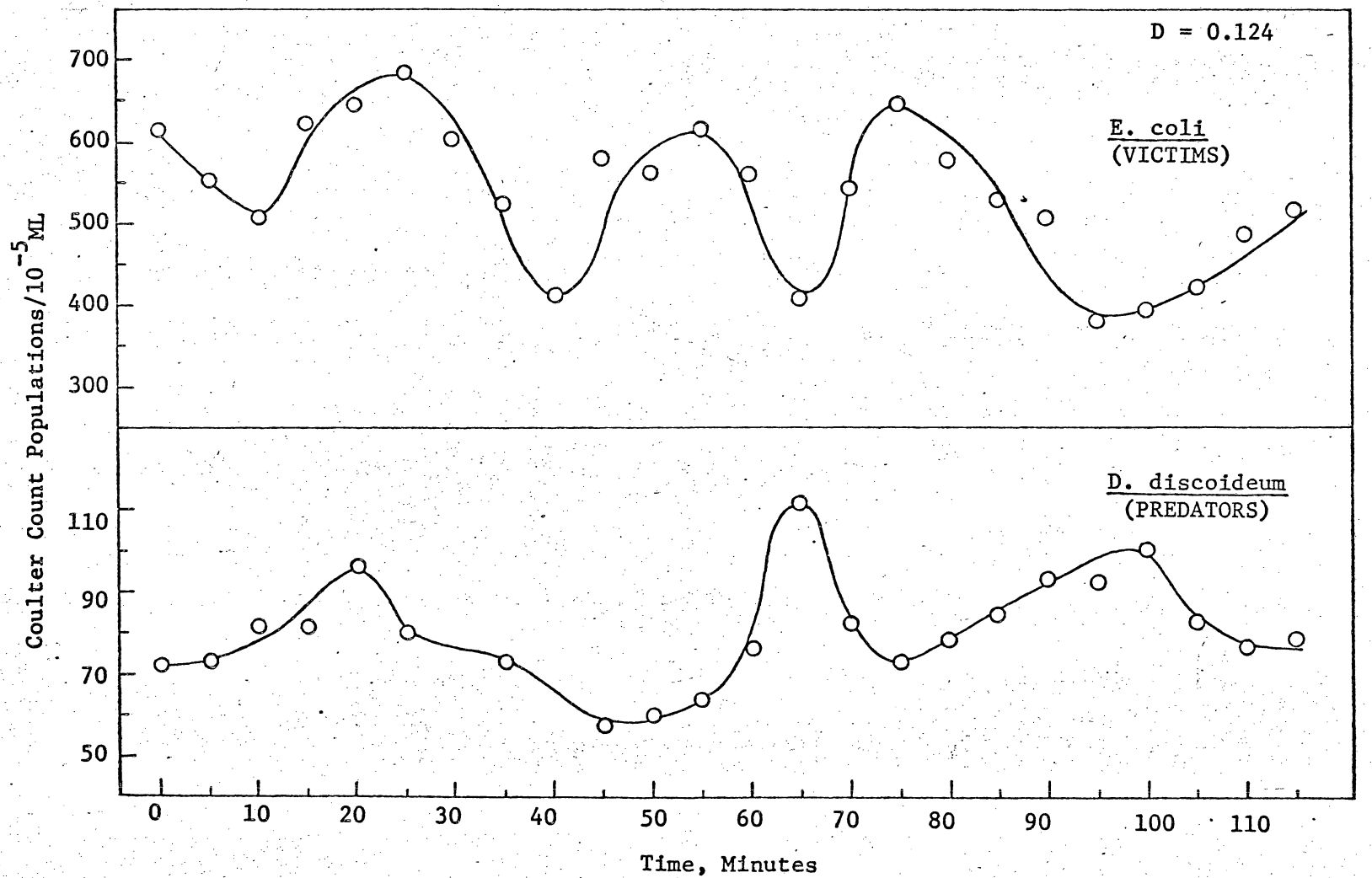

Figure 7. Fluctuation Populations in E. coli and D. discoideum Interaction

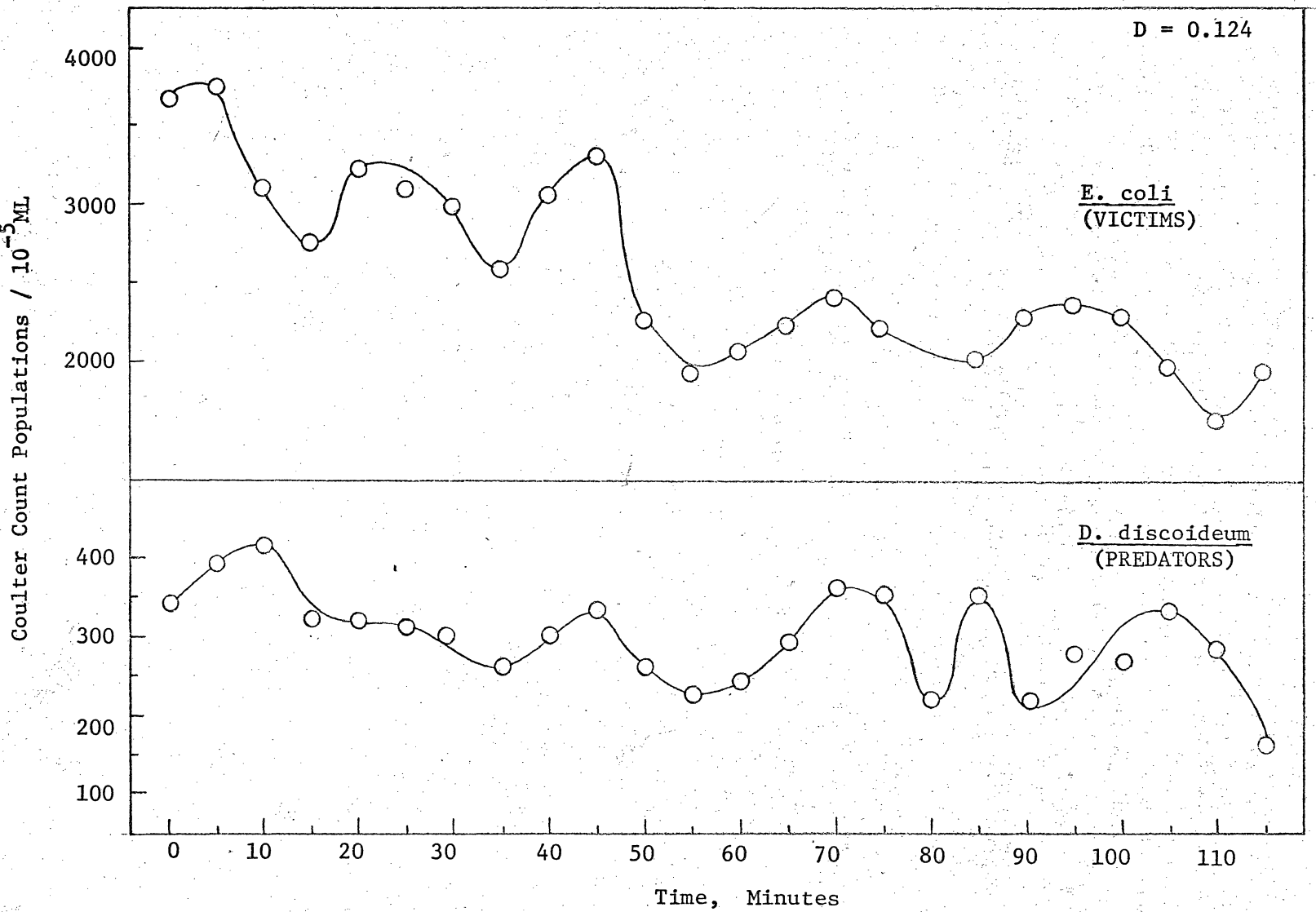

Figure 8. Fluctuation Populations in E. coli and D. discoideum Interaction

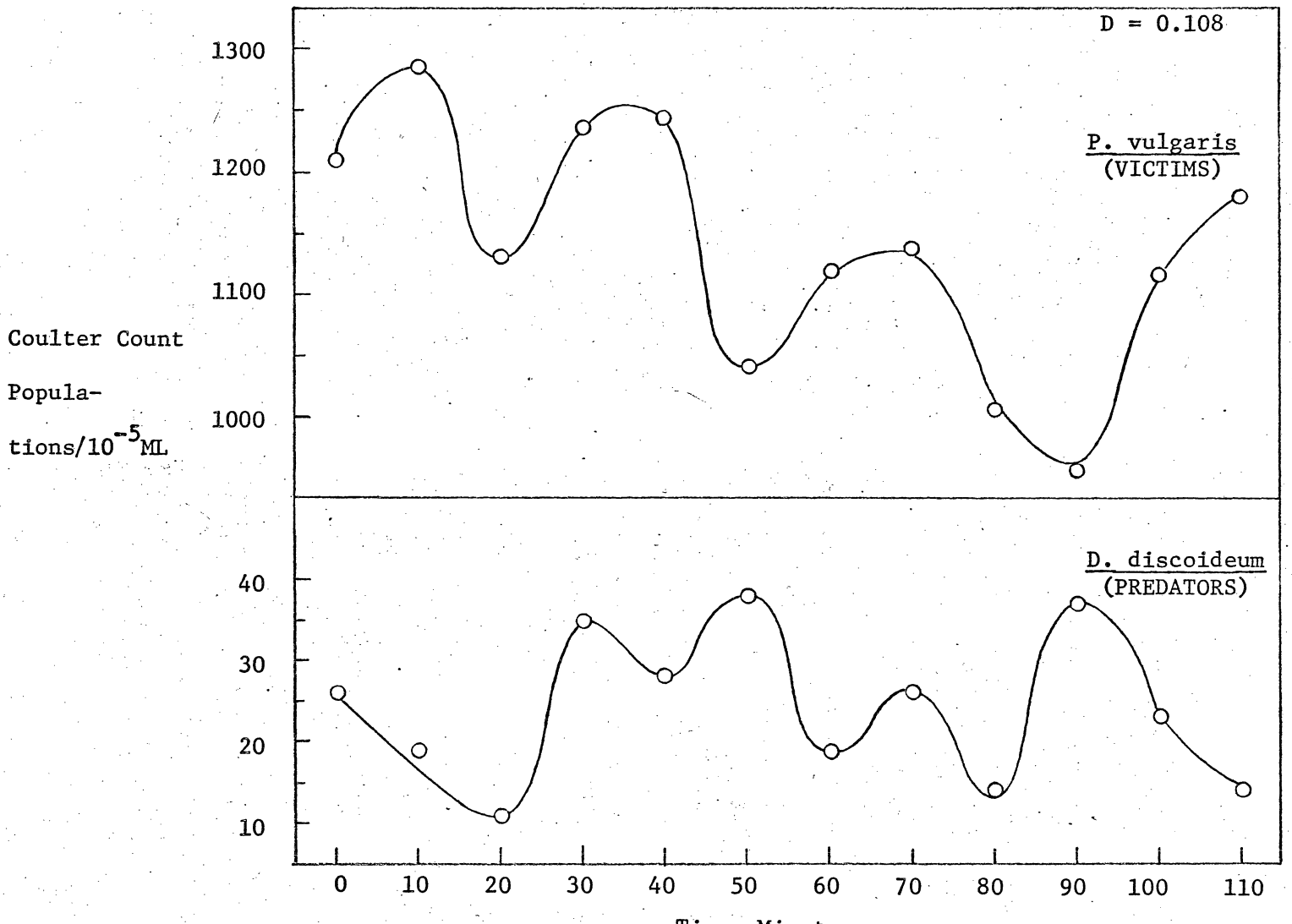

Figure 9. Fluctuation Populations in P. vulgaris and D. discoideum Interaction

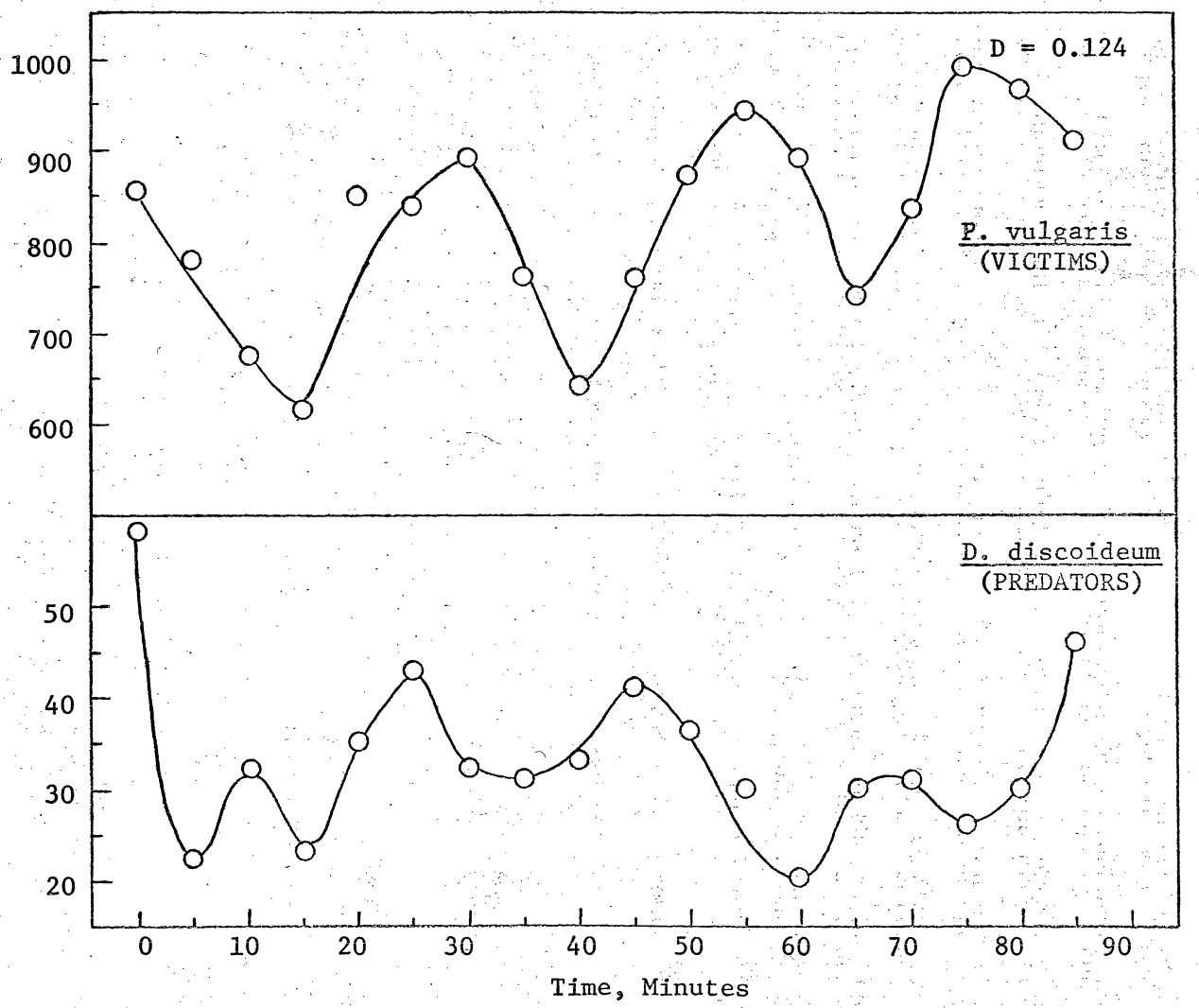

Figure 10. Fluctuation Populations in P. vulgaris and D. discoideum Interaction

Table 3. Comparison of Coulter Counter Counts with Petroff-Hausser Counts

Sample	Coulter Counter		Petroff-Hausser	
	E. coli	P. vulgaris	E. coli	P. vulgaris
1	7.6×10^6	8×10^6	68×10^6	90×10^6
2	11.4×10^6	5.3×10^6	80×10^6	80×10^6
3	15.8×10^6	9.4×10^6	86×10^6	110×10^6

In these experiments, runs lasted from one to two weeks before being terminated by contamination or by mechanical failure. Air flow was adjusted to a reasonable rate but not measured until the end of experiments. A typical air flow rate was roughly 60 ml. per minute.

DISCUSSION

Population oscillations occurred in a continuous flow culture of either bacterial species with myxamoeba (*E. coli* - *D. discoideum* and *P. vulgaris* - *D. discoideum*). The period of oscillations was approximately 25 to 35 minutes. Different dilution rates did not cause a measurable change in the period or amplitude of population oscillations.

The population levels of *E. coli* or *P. vulgaris* in continuous pure culture decreased linearly with increasing dilution rates. This disagrees with the continuous culture theory in that the population level should remain fairly constant over a series of dilution rate changes and then decrease suddenly at a certain critical dilution rate.

Proper and Garver (1966) studied a protozoan-bacterium interaction and explained that the rate of growth of the protozoan (predator) is limited by the rate of ingesting bacteria (prey) and the maximum rate of metabolism of bacteria. By similar reasoning the rate of growth of myxamoeba will depend on the rate of ingesting bacteria and the rate of metabolism of bacteria. A high population of bacteria provides sufficient food for rapid reproduction of myxamoeba, which results in the bacterial population being lowered. The reproduction of myxamoeba declines because of the lower bacterial population and removal by wash-out from the vessel gives a reduction in predation. This permits the bacteria to reproduce quickly and regain their former high population level which will cause more frequent contact between the two species and the cycle is again repeated. An analysis of this mechanism shows that the period of oscillation of myxamoeba should be the same as the period of oscillation of bacteria.

Sources of error in these experiments were clumping, wall attachment, growth on the walls of the effluent tube and simultaneous passage through the orifice. Shorter sample time intervals would give better definition of the population curves. For future research work, the effects of dilution rate changes on the period and amplitude of population oscillation should be further investigated, and an analysis of effects of pH and temperature variations on this population oscillation should be made.

CONCLUSION

1. Population oscillations occured in the prey-predator systems of *Escherichia coli* - *Dictyostelium discoideum* and *Proteus vulgaris* - *Dictyostelium discoideum*.
2. The period of oscillations was approximately twenty-five to thirty-five minutes.
3. Different dilution rates did not cause a significant change in the period or amplitude of population oscillations.

SUMMARY

An investigation was made on the prey-predator systems Escherichia coli - Dictyostelium discoideum and Proteus vulgaris - Dictyostelium discoideum. An attempt was made to characterize the relationship in terms of population oscillations expected to occur in the prey-predator interaction and also to investigate the effects of dilution rate on the population oscillations.

A continuous flow apparatus and commercial lactose broth and tryptic soy broth were used. A dilute medium was selected in order to obtain low bacterial populations to prevent the accumulation of products toxic to the myxamoeba and insure that aeration will not be a limiting factor. A Coulter Counter was used to enumerate the population levels and checks were made periodically with a Petroff-Hausser bacterial counter. Effluent samples were collected at either five and ten minute intervals for counting.

Population oscillations occurred in both prey-predator systems in continuous flow culture. Ingestion by myxamoeba lowers the bacterial population until it limits the predator food supply to cause a decline in numbers of predators. Less predation permits the bacteria to reproduce and regain their former high population. The repeat of cycle produces sustained population oscillations. The period of oscillation was approximately 25 to 35 minutes. Different dilution rates did not lead to a significant change in the period or amplitude of population oscillations.

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POPULATION OSCILLATION IN CONTINUOUS CULTURE OF THE PREY-PREDATOR SYSTEMS *ESCHERICHIA COLI* - *DICTYOSTELIUM DISCOIDEUM* AND *PROTEUS VULGARIS* - *DICTYOSTELIUM DISCOIDEUM*

by

Shin Jong Chen, B.S.

ABSTRACT

An investigation of expected population oscillations in a continuous flow culture of prey-predator interactions was made. A Coulter Counter was used to enumerate the population levels and periodic checks were performed with a Petroff-Hausser bacterial counts.

Population oscillations of both prey and predator occurred in the continuous flow culture of either bacterial species with the myxamoeba. Ingestion by myxamoeba lowers the bacterial population until it limited its own food supply to cause a decline in its population. Fewer predators permit the bacteria to reproduce and regain their former high population. The repeat of cycle produces population oscillations. The period of oscillation was approximately 25 to 35 minutes. Different dilution rates did not lead to a significant change in the period or amplitude of population oscillations.