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The Dispersal and Dispersion Patterns of Hydra Fusca in a Limited Environment

Faith E. Ruffing
Portland State University

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AN ABSTRACT OF THE THESIS OF Faith E. Ruffing for the Master of Arts in Biology presented June 28, 1977.

Title: The Dispersal and Dispersion Pattern of Hydra fusca in a Limited Environment.

APPROVED BY MEMBERS OF THE THESIS COMMITTEE:

Richard Petersen, Chairman

Robert O. Tinnin

Leonard Simpson

The dispersal and dispersion patterns of Hydra fusca were examined. Hydra were placed in petri dishes at various densities. The water in the dishes was swirled forcing the animals to the center. The location of each animal was marked at time intervals thereafter. Analyses of the dispersal rates and the dispersion patterns were made. Hydra dispersed from a central release point at a non-random rate. There was rapid movement from the center followed by a min-

imal daily movement. This eventually resulted in a uniform dispersion pattern at high densities in a limited environment. There was a relationship between the ratio of nearest neighbor distances to expected distances and the density. The inhibition of growth with an increase in density was demonstrated. The decrease of density through distribution of the polyps in a uniform pattern could be related to the release of a growth inhibiting substance into the medium.

THE DISPERSAL AND DISPERSION PATTERNS OF HYDRA FUSCA
IN A LIMITED ENVIRONMENT

by

FAITH E. RUFFING

A thesis submitted in partial fulfillment of the
requirements for the degree of

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in
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TO THE OFFICE OF GRADUATE STUDIES AND RESEARCH:

The members of the committee approve the thesis of
Faith E. Ruffing presented June 28, 1977.

Richard Petersen, Chairman

Robert O. Tinnin

Leonard Simpson

APPROVED:

Herman Taylor, Head, Department of Biology

Stanley E. Rauch, Dean of Graduate Studies and Research

Juniper

DEDICATION

This thesis is dedicated to Janice M. Ruffing, the author's sister. Without her encouragement and support over the years, this thesis and degree would not have had a beginning.

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CHAPTER I

INTRODUCTION

Hydroids have been reported by several authors to release a substance into the surrounding waters which subsequently controls the growth of the organism. Rose (1940) described a "regeneration inhibiting substance" released by Tubularia tissues. Fulton (1959) suggested that this substance was in fact due to bacteria or their metabolic by-products. Davis (1966) reported inhibition of growth and regeneration in Hydra by water from crowded cultures. He eliminated the possibility of the causative agent being ammonia or bacteria and concluded that it was a molecule in the 5,000 to 10,000 molecular weight range. In further studies with homogenized hydra, he concluded that the inhibitor was nematocyst toxin (1967). Ruffing (1967) reported inhibition of regeneration in Hydra viridis using the culture water from crowded H. pseudoligactis. More recently, Thorpe (1975) demonstrated an inverse relationship between fixed densities and growth rate in H. viridis. He also demonstrated a decrease in asexual reproduction rates in animals grown in culture water from either crowded or uncrowded cultures. These rates were significantly different from animals growing in fresh culture medium. He attributed the

difference to a water borne substance in the culture water.

Both the inhibition of regeneration and the decrease in growth rate demonstrate responses of the organism to population density. The density is determined through the feedback mechanism of the inhibiting substance released into the water. This control mechanism could also be used to regulate the density without the curtailment of growth, if the population is able to expand.

Although Hydra are sedentary most of the time, they have developed different modes of locomotion. Some modes, such as actively gliding along the substratum, are relatively slow. Others, such as the somersault or inchworm modes, are quite rapid and, in fact, the hydra can move several centimeters in a few minutes. This mobility can alter the spatial arrangement of individuals within the populations. Both dispersal rate and pattern can be measured and correlated with population density.

By means of the inhibitor feedback mechanism, the hydra could move from the more densely populated areas to less dense areas. This movement would be non-random because the movement would be in response to the gradient created by the concentration of the inhibitor in the area with more animals. This movement would eventually result in a uniform dispersion of the organisms if the environment of the hydra was limited and the density was sufficient to spread the organisms over the entire space.

Measurement of the dispersal rates of living organisms from a central release point has been described by Pearson (1906) and reported in Skellam (1951) and Poole (1974). The square root of the mean square radial distance ($MSD^{\frac{1}{2}}$) over time will increase linearly if the organisms are dispersing randomly. Also, the dispersion pattern of the organisms can be predicted (Clark and Evans 1954). The nearest neighbor distances are used to determine whether the organisms are dispersed in a clumped, random or uniform pattern.

The research reported here is concerned with: (1) measuring the dispersal rates of Hydra from the center of the petri dish, (2) the determination of the dispersion patterns in this limited environment, and (3) the effect of density on the dispersal rates and patterns. If the dispersal rate is non-random and the pattern tends toward uniformity at high density, the organisms could be responding to a mechanism for controlling population density. The exact nature of this mechanism and its relationship to the inhibiting substance described by the above authors should be the topic of future work and will not be covered by this thesis.

CHAPTER II

MATERIALS AND METHODS

Hydra fusca were cultured according to the method of Loomis and Lenhoff (1956) except that this author used distilled water rather than tap water. Polyyps were randomly distributed in 95 mm petri dishes holding 125 ml water. Animals with at least a stage one bud were selected after feeding.

PART 1

Triplicate sets of dishes were prepared at densities 10, 40 and 80 animals per dish. The water was swirled, forcing the animals to the center of the dish. The position of each animal was marked on a grid of 1 mm squares at 0, 3, 24 hours and daily thereafter for a total of seven days. The hydra were not fed throughout this experiment and the water was not changed. Water lost through evaporation was replaced to keep the water level at the top of the dish.

PART 2

Animals were acclimated to a constant density of 10, 50 and 100 animals per dish for 10 days by removing the detached buds daily. This was followed by a maintenance peri-

od of 13 days when the number of budding animals was recorded. The animals were swirled to the center on the 20th day. The position of the animals was recorded from the 17th to the 23rd day.

Throughout the 23-day period the animals were fed on alternate days and the water was changed daily by carefully pouring off the medium and replacing it so as not to disturb the animals, except on the 6th, 10th, 16th and 19th days when the animals were transferred to clean dishes.

CHAPTER III

RESULTS

PART 1

The animals were swirled to the center and the positions were noted for seven days without fresh medium.

The mean square radial distance, MSD, for each dish was calculated according to

$$MSD = \frac{\sum r^2}{n} \quad 1$$

where n is the number of animals in each dish and r is the distance of each individual from the center. The square root of the MSD for each dish was averaged. The results are summarized for each day in Table I and graphically represented in Figure I.

The distribution pattern was determined using the nearest neighbor evaluations described by Clark and Evans (1954). The distance between each organism and its nearest neighbor was determined. If n is the number of observations, the observed mean distance is

$$\bar{r} = \frac{\sum r}{n} \quad 2$$

If the dispersion pattern is random (Poisson), the ex-

TABLE I

DISPERSAL RATES

HOURS	DENSITY		
	10/dish	40/dish	80/dish
0	6.1	7.5	8.3
3	24.1	16.1	23.7
24	27.3	21.3	25.2
48	27.0	23.3	26.5
72	27.2	25.2	27.6
96	30.9	27.9	30.8
120	31.2	30.2	31.8
144	33.1	30.3	34.7
168	31.8	31.2	35.9

Figure 1. Dispersal rates as determined by the $MSD^{1/2}$ per unit time. Average of three dishes for each density.

pected value of r is

$$E(r) = \frac{1}{2\rho^{1/2}} \quad 3$$

where ρ is the density.

For a random (Poisson) dispersion pattern, the ratio, R , will be close to 1.

$$R = \frac{\bar{r}}{E(r)} \quad 4$$

Significant deviation from $R = 1$ indicates a clumped or uniform pattern depending on the direction of the deviation. If the individuals are clumped, $\bar{r}$ will be small, and $R < 1$, while if the individuals are uniformly dispersed, $\bar{r}$ will be large and $R > 1$. The significance of the deviation may be tested by

$$Z = \frac{\bar{r} - E(r)}{SE(r)}, \text{ where } SE(r) = \frac{.26136}{(n\rho)^{1/2}} \quad 5,6$$

and Z is the standard normal statistic.

The Z values are summarized in Table II and Figure II. Those < -1.96 indicate a clumped pattern; those between ± 1.96 indicate a random pattern and those Z values > 1.96 indicate a uniform pattern, at the 95 per cent level of confidence.

PART 2

The growth rate for the animals was determined from the per cent budding animals and the per cent increase as determined by the number of buds removed each day. The mean

TABLE II

Z VALUES FOR DETERMINATION
OF DISPERSION PATTERNS

HOURS	DENSITY		
	10/dish	40/dish	80/dish
0	-48.16	-24.70	-18.34
3	-27.03	-17.17	-11.50
24	-25.34	-15.37	-8.53
48	-21.30	-12.09	-9.88
72	-16.50	-11.47	-5.56
96	-3.91	-2.36	-0.03
120	-9.24	-1.66	+1.40
144	-5.31	+0.81	+2.05
168	-11.14	+1.45	+1.28

Figure 2. Dispersal patterns as determined by the Z values per unit time. Average of three dishes for each density. Patterns are clumped for the Z value below -1.96, random for values between ± 1.96 , and uniform for values above 1.96.

value for each density is given in Table III. The relationship between the density and the growth rate is diagrammed in Figure III.

The dispersal of the animals from the center of the dish was calculated for the 21st and 23rd days. The $MSD^{1/2}$ is shown in Table IV and Figure IV.

The pattern of dispersion was analyzed for the 17th, 19th, 21st and 23rd day of the experiment. The first two days correspond to 24 and 72 hours after a haphazard placement of the animals in clean dishes. The latter correspond to 24 and 72 hours after placement in clean dishes immediately followed by swirling to the center. The results are shown in Table V.

TABLE III

GROWTH RATE VS. DENSITY

	DENSITY		
	10/dish	50/dish	100/dish
MEAN % BUDDING	59.42 $\pm$ 11.4	44.0 $\pm$ 6.21	34.42 $\pm$ 3.45
MEAN % INCREASE	36.67 $\pm$ 22.26	26.83 $\pm$ 11.0	16.0 $\pm$ 6.19

Figure 3. Growth rate vs. density. Growth rate expressed as per cent budding and percent increase. Average of six dishes.

TABLE IV

MSD^{1/2} VS. DENSITY

HOURS	DENSITY		
	10/dish	50/dish	100/dish
24	26.75	33.6	32.6
72	31.6	33.6	34.3

Figure 4. Dispersal rates of hydra conditioned to specific densities. Average of six dishes.

TABLE V

DISPERSION PATTERNS OF
ANIMALS CONDITIONED TO
SPECIFIC DENSITIES

DAY	PLACEMENT	DENSITY		
		10/dish	50/dish	100/dish
17	Haphazard	-8.690	+0.949	+2.167
19	Haphazard	-1.489	+0.548	+1.272
21	Swirled	-19.080	-3.148	-2.251
23	Swirled	-11.384	-0.251	+0.429

CHAPTER IV

DISCUSSION

The dispersal of the hydra from the center of the dish is non-random over time. Animals moving in a random fashion would have the same $MSD^{\frac{1}{2}}$ each day. In the experiments performed here, there is a rapid dispersal from the center in the first few hours. This is followed by a seven day period in which there is little outward movement. In animals conditioned to specific densities, the non-random movement from the center also is observed.

The density dependence of the dispersal is clear for the upper two densities. That is, the more crowded animals move further away initially and then maintain this difference for the next seven days. In the first experiment, the low density animals moved further out than the animals at the highest density. This may be a statistical artifact due to the low number of animals in the dish. In the second part, the dispersal is clearly density dependent.

The dispersion patterns of the animals indicate that the polyps will shift their positions to reduce the density. After the initial outward dispersal, farther movement resulted in increasing the distance between the animals in the dish rather than the distance from the center. This dis-

tance between nearest neighbors increased with time at all densities. The ratio, R , increased with density and this increase was reflected in the Z values. Eventually the animals moved so that the Z values indicated a trend toward a clumped, random and uniform pattern for the low, medium and high densities. In the second part, the animals acclimated to the densities demonstrated these patterns after 24 hours. These were not maintained at 72 hours although the relative distances were still density dependent. When these animals were swirled to the center, the clumped pattern was displayed after 24 hours for all the densities, but by 72 hours the higher densities had attained a random dispersion pattern.

The density dependence of the dispersal rate would support a feedback mechanism for determining the population density. Presumably, the intensity of the stimulus for density would be greater for the animals in the high density and they would therefore move farther away from the center. Once away from the strong stimulus in the center of the dish, the stimulus would become much less and the movement curtailed. As time proceeds, the buildup of the inhibiting substance in the water would stimulate the animals to move away from one another. Again the stimulus would be stronger in the high densities resulting in the more uniform pattern development.

There are at least two possibilities other than the inhibiting feedback mechanism which would result in the movement of the hydra. One is the need for food and the result-

ant searching movement. The other is a tactile stimulus. This author concluded that the stimulus is probably not tactile for two reasons. First, the movement of the animals out of the center is very rapid, entailing the somersault mode of locomotion. In this mode the animal bends over, attaches its tentacles to the substratum, detaches the base, flips the proximal end over and reattaches the base to the substratum. The tentacles are then detached and the distal portion is flipped over, the tentacles reattach and the process is repeated. All of this action occurs in a few minutes, propelling the animal several centimeters away from the center. There was no waving of tentacles or other movements to detect the other animals during the somersaulting action. Secondly, animals have been observed within a few mm of each other. Since the tentacles of these hydra are at least 5 mm the animals are well within the range of the tentacle swing. If the dispersal of the animals was a result of tactile stimulus, one would expect the animals to keep at least a tentacle length distance from one another.

A need for food would be a possible stimulus for the animals to move in the first part. The animals were not fed for several days and at least some of the movement could be attributed to the search for food and fresh water. However, this would not explain the density dependence of the dispersion patterns. Furthermore, in the second part the animals were well fed, yet the dispersal from the center of the dish

and the subsequent shift in the pattern was still density dependent.

There are two portions of data that do not seem to support an inhibitor feedback mechanism. The first is the fact that in both sets of experiments, the dispersion did not remain uniform once uniformity had been attained. This may be due to several factors, such as a decrease in the growth rate and therefore a decrease in the inhibitor concentration, the death of some individuals and the replacement by detached buds which would be closer to the parent animal, or death of some individuals and a reduction in the amount of inhibitor therefore reducing the stimulus to keep at a distance. None of these possibilities is resolvable with the data presented here.

The other aspect of the data which does not quite fit the inhibitor feedback mechanism is seen by comparing the two parts of the experiment. The animals in the second part attained appropriate dispersion patterns much more quickly than those of the first part although the water was changed daily preventing a build up of the inhibitor. The fact that the animals were acclimated to the densities may somehow explain this, but it is not exactly clear how this would affect the inhibitor production.

This ability of the hydra to move in response to their density provides them with a mechanism whereby a density within a certain range can be maintained. That is, animals

that are too crowded or close together tend to move away from one another. They move until a lower limit of density has been obtained. If the density of the animals is sufficiently high, the lower limit will only be obtained when the animals are as far from one another as possible and a uniform pattern will emerge.

There are several possible advantages to the hydra for keeping the density within a certain range. Decreasing the density from a clumped situation spreads the animals over more territory increasing the food gathering area, prevents the buildup of waste products such as ammonia and carbon dioxide to intolerable concentrations and allows them to increase the population from a variety of points instead of only at the edge of the culture. Each hydra producing offspring asexually becomes a center of population growth. As the polyps detach and mature, they move away from the parent until an uncrowded area is found.

On the other hand, maintaining a lower limit on the density could be helpful in decreasing the physiological stress to the individual. Although adapted to fresh water, the hydra are not isotonic with their environment. They must constantly fight the influx of water into their cells by the expulsion of the gut contents through periodic contractions of the gastric region. If the density is not allowed to fall below a certain lower level, the concentration of dissolved materials would be increased in the immediate vicinity of the

group. This would alter the micro-environment of the organisms and a more suitable environment would be obtained with less work for the individual.

A further advantage of limiting the lower density would be to insure that the animals are close enough for cross fertilization in the fall and the production of encysted egg to carry the species through the winter.

CHAPTER V

CONCLUSION

The data in this thesis present significant evidence that hydra will respond to the density of the population by moving to less dense areas. This response is characterized by the non-random movement out of the center of the dish followed by the eventual development of specific dispersion patterns which are directly related to the density, i.e., the more dense the population the more the patterns tend toward uniformity. This phenomenon is observed in conjunction with a decrease in the growth rate with density increase. It is viewed by the author as a mechanism which serves to enhance the fluctuation of the growth rate to control population density.

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