*Ecology* (1975) **56**: pp. 206–212

# EFFECTS OF CROWDING ON GROWTH RATE AND SYMBIOSIS IN GREEN HYDRA[1]

JAMES H. THORP III[2] AND GEORGE T. BARTHALMUS
*North Carolina State University, Raleigh, North Carolina 27607*

*Abstract.* In order to examine the relationship between crowding and growth rate in green hydra (*Hydra viridis*), we raised animals at various levels of fixed population densities; all new individuals in excess of the fixed densities were counted and removed every 4 days. A significant inverse relationship between population density and population growth rate exists. In addition, hydras were found to increase or decrease their growth rates in response to rapid changes of density after acclimation to fixed densities. The most dramatic effects were noted when the changes in degrees of crowding were greatest.

Investigation of the effects of crowding in hydras on the total quantity of algal endosymbionts and their pigments revealed no significant changes in algal numbers or in carotenoid to chlorophyll-*a* ratios at any level of crowding.

Data on the presence of a water-borne inhibitor of asexual reproduction supported the hypothesis that crowded culture medium contains a substance which depresses growth rates in green hydras.

*Key words: Algae; crowding; growth inhibitors; growth rate; hydra; pigment ratios; symbiosis;* Zoochlorella.

## INTRODUCTION

The population growth rates of some aquatic organisms, like many terrestrial species, are depressed by high population densities. In many cases a thorough analysis of the nature and impact of crowding has not been undertaken. Our study was designed to (1) confirm the inverse relationship between the asexual population growth rates (subsequently called growth rates) of green hydras, *Hydra viridis*, and their population densities; (2) determine the impact of rapid changes of densities on this rate; (3) investigate possible quantitative changes of algal cells and their pigments in relation to density stresses on the hydra; and (4) determine the effect on the growth rate of water-borne inhibitors produced by the hydra.

The importance of population density in the ecology of many organisms is well established. Studies with *Daphnia* (Frank et al. 1957) indicate that increased density is accompanied by a decreased birth rate, increased survival rate, and minimal changes in the death rate. Additionally, crowding affects various biological activities of hydra (Loomis 1956, Loomis and Lenhoff 1956, Park et al. 1961, Davis 1966, Lomnicki and Slobodkin 1966). Loomis (1954) observed that as the density of a culture of brown hydra was allowed to increase progressively, growth rates declined. Yet Lytle et al. (1971) reported that the effect of previously crowded conditions on growth rates varied considerably between two species of green hydra (*Chlorohydra viridissima* and *C. hadleyi*). Past studies have neither determined the effect of various levels of crowding on the growth rates of continuously maintained densities of hydra, nor indicated evidence of the impact of rapidly changing densities on these rates.

The taxonomic status of the algal endosymbionts of green hydra appears questionable (Oschman 1967). They have been referred to as *Zoochlorella conductrix*, *Chlorella conductrix*, or *Chlorella* sp., but are generally referred to as zoochlorellae. Zoochlorellae both augment the survival of their hosts (Muscatine 1965, Muscatine and Lenhoff 1965, Smith et al. 1969) and benefit from their symbiosis (Muscatine and Lenhoff 1965, Cook 1972). Since this relationship appears mutualistic, stresses on the hydra may be reflected by qualitative and/or quantitative changes in the endosymbionts. Our study investigates changes in numbers of algae per packed volume of hydra, in quantities of chlorophyll and carotenoids, and in the ratio of these pigments extracted from the zoochlorellae.

Finally, this study examines the effect of potential water-borne inhibitors on the growth rate of the green hydra. The existence of a water-borne inhibitor has been suggested by others (Brown 1946, Rose 1959*a*, *b*, Davis 1966, Lomnicki and Slobodkin 1966).

## MATERIALS AND METHODS

*Hydra viridis* (synonymous with *Chlorohydra viridissima*: Oschman 1967, Smith et al. 1969) was collected in the spring and summer of 1972 from a

[1] Manuscript received January 10, 1974; accepted June 10, 1974.

[2] Present address: National Marine Fisheries Service, Atlantic Estuarine Fisheries Center, Beaufort, NC 28516.

pond in Raleigh, North Carolina. All hydras used in the experiments were derived from their asexual generations. Cultures were grown in M-solution (Muscatine 1961).

On alternate days, the hydras were fed excess *Artemia* spp. nauplii for 1 h. Hydras were maintained in a growth chamber of the North Carolina State University Phytotron at a constant water temperature of 18° ± 0.5°C and under 12-h fluorescent illumination (1,000 ft. candles). All animals were kept in 8-cm (inner diameter) culture dishes with 40 ml of culture solution. Cultures were cleaned and the medium was changed on feeding days. Unless specified, we maintained populations at constant densities by counting hydras every 4 days and removing excess individuals. The change in numbers is expressed as new individuals per adult hydra per 4-day period, which is equivalent to a population growth rate. Since purely somatic growth in hydras results in an increased number of hydras in the culture, rather than an increased size of an individual (Loomis 1954), counting hydras is an accurate measure of their growth rate. At the beginning of each experiment the hydras were randomly selected from stock cultures and added to the experimental culture dishes.

*Effects of fixed density on growth rates*

We examined the effects of crowding on growth rates by maintaining populations at densities of 20, 70, 120 and 170 hydras per 40 ml culture solution. Initially, at least seven replicates of each density were cultured under standard conditions. At intervals, some of the replicates were removed (at least three remained at the end of the experiment) and quantitative changes in the algal cells and pigments were measured. Replicates with a decline in density of 10% or greater were eliminated. For convenience, the hydras used to maintain fixed densities were referred to as "adults." Generally, they were larger than those hydras designated as "new individuals" which were counted and removed every 4 days.

*Effects of rapid changes in population densities*

To analyze further the relationship between crowding and asexual reproduction we acclimated hydras to fixed densities (treatment 0) and then transferred them to greater or lesser population densities (treatment 1). Hydras were acclimated for 16 days to either 20 (uncrowded) or 160 (crowded) hydras per 40 ml of culture solution. In treatment 1 dishes of uncrowded hydras were combined to either 80 or 160 hydras or allowed to remain at 20 hydras per dish (control) for 12 days. Concurrently, dishes of crowded hydras were divided into replicates of 20 or 80 hydras, or kept as controls at 160 hydras per dish. The number of replicates for each density varied (between 4 and 7) during the acclimation period since varying numbers of hydras were required to begin treatment 1 (e.g., 32 dishes of hydras were required to produce 4 replicates of 160 hydras while only 16 dishes were necessary to produce 4 replicates of 80 hydras per dish). Again, animals were maintained under standard conditions and dishes of hydras were removed at specific intervals for analysis of the zoochlorellae.

*Effects of crowding in hydra on endosymbionts*

Qualitative and quantitative changes in the endosymbionts in response to density stresses on their hydra host were investigated. As noted earlier, at intervals cultures of hydras were withdrawn and their endosymbionts were removed, counted, and spectrophotometrically analyzed for pigment content. To extract the algal cells, we centrifuged the hydras for 1 h at speed #5 of the I.E.C. International Clinical Centrifuge, and the packed hydra volume was recorded to the nearest five-thousandth of a milliliter. This step was necessary because the volume of individual hydra varies. The hydras were solubilized in 15% Triton X-100®. Three hemocytometric samples per test tube (5 ml) of solubilized hydras were taken and the algal cells were counted by means of a Spencer Bright-Line Hemocytometer. The solutions of solubilized hydras containing whole algal cells were then filtered through 0.45-$\mu$ Millipore membrane filters and washed with 10 ml of distilled-deionized water and 2 ml of magnesium carbonate suspension.

We performed a spectrophotometric analysis in darkness of chlorophyll, carotenoids, and phaeo-pigments using a modified technique of Strickland and Parsons (1968). The Millipore filters were partially dissolved in 2 ml of 90% spectranalyzed acetone and ground with a tissue grinder for 3 min at 1,600 rpm. The volume then was brought to 5 ml, after which the samples were refrigerated in the dark for 20 h. The samples were then readjusted to 5 ml and allowed to warm to room temperature. After centrifugation for 10 min at 4,000 rpm, the supernatant was decanted into 1 cm light path, silica cuvettes. The extinction coefficients were recorded at 430, 480, 630, 645, 665 and 750 nm with a Beckman-DU Spectrophotometer and expressed as milligrams of pigments per packed volume of hydras.

Corrections were made for "cell-to-cell" variations of the sample cells against the reference cell, and for non-pigment turbidity absorptions. Final calculations of pigment concentrations were adjusted to their equivalents in 10 cm cuvettes with 10 ml of acetone.

Concentrations of pigments were calculated from

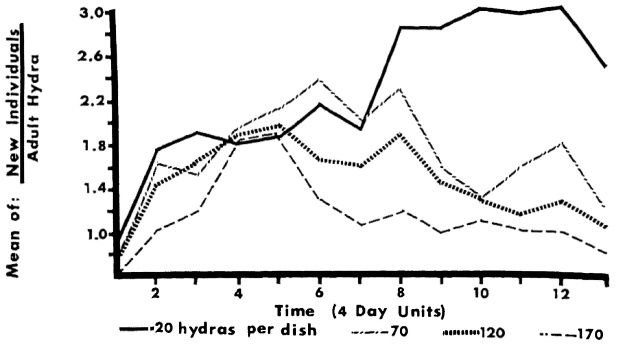


FIG. 1. Effects of fixed levels of crowding on growth rates in green hydras.

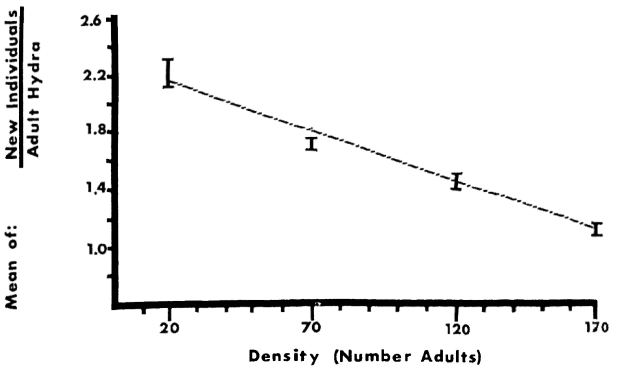


FIG. 2. Predicted growth rates under crowded conditions. Horizontal bars represent SE of $\bar{Y}$.

equations modified from Strickland and Parsons (1968):

$$\text{mg (or m-SPU) pigment/mm}^3\ \text{PHV} = C/V,$$

where $V$ is the packed hydra volume (PHV) in mm$^3$, m-SPU is millispecific plant unit (approximately 1 mg of dry pigment), and $C$ is a value obtained from the following equations ($E$ stands for extinction values):

$$C\ (\text{chlorophyll } a) = 11.64E_{665} - 1.31E_{645} - 0.14E_{630}$$
$$C\ (\text{Chlorophyta plant carotenoids}) = 4.0E_{480}.$$

*Investigation of water-borne inhibitors*

A final experiment was designed to investigate the effects on asexual reproduction of possible water-borne inhibitors. Four replicates of crowded hydra dishes (160 hydras per dish) were acclimated under standard conditions for 12 days (treatment 0). The replicates then were pooled and subsequently divided into 16 dishes of 20 hydras per 40 ml. Similar to Davis (1966), test groups included (1) FCM, hydras in fresh culture medium; (2) CCM, hydras in crowded culture medium; (3) HCCM, hydras in heated CCM; and (4) UCCM, hydras in uncrowded culture medium. Culture medium was obtained from 2-day-old crowded medium (160 hydras per dish) and passed through untreated coarse filter paper. Half of this medium served as CCM; the other half was first boiled for 2 min (destroying any proteins which might be present), cooled to room temperature, and then used as HCCM. UCCM was obtained from 2-day-old uncrowded culture medium (20 hydras per dish). The growth rates of these groups (treatment 1) were recorded for 12 days.

Growth rates for all experiments were compared by the analysis of variance procedure and Duncan's Multiple Range test as programmed in the Statistical Analysis System (SAS) of the North Carolina State University's IBM 360 computer. The SAS compared (1) slopes of population lines, (2) mean growth rates, and (3) time-population interactions (Time*Pop.) i.e., the effect of time on each population.

## RESULTS

*Effects of fixed density on growth rates*

An inverse relationship exists between fixed population density and population growth rate. Figure 1 illustrates the effects of crowding on growth rates (mean number of new individuals per adult hydra), where each point designates the average growth rate for replicates at a fixed density. Growth rates peaked after 16–20 days in dense populations (120 and 170 hydras per dish), after 24–32 days at an intermediate density (70 hydras per dish) and after 32–48 days at the lowest density (20 hydras per dish).

The mean growth rate for every replicate was calculated over time and plotted against initial numbers of adult hydras. The population regression line in Fig. 2 represents the mean predicted value for growth rates at each density. The standard error of $Y$ indicates that the actual data conform closely to predicted values. An analysis of the slope of the population regression line suggests that as crowding increases asexual reproduction decreases ($t$-test, $P < 0.0001$).

*Effects of rapid changes in population densities*

Results of these experiments support the conclusions of the fixed-density experiment and also indicate that hydras are affected by rapid changes in population densities. Changes in growth rates were most dramatic when population densities were altered by the greatest amount (e.g., when population densities are increased from 20 to 160 hydras or decreased from 160 to 20 hydras per dish).

*Effects of rapidly increased population densities.*—Although the three hydra populations in treatment 0 (Fig. 3) were of equal density, the slopes of their lines are significantly different ($F$-test: $P < 0.05$). However, the mean growth rates of these populations

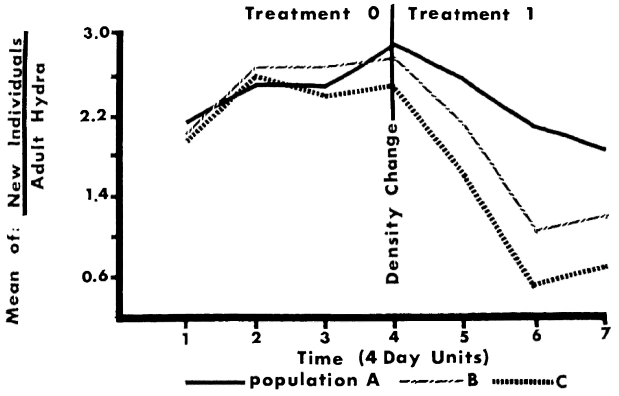


FIG. 3. Effects of abrupt increases in crowding on growth rates in green hydras. All populations in treatment 0 are at densities of 20 hydras per dish. In treatment 1, populations A, B, and C are at densities of 20, 80, and 160 hydras per dish, respectively.

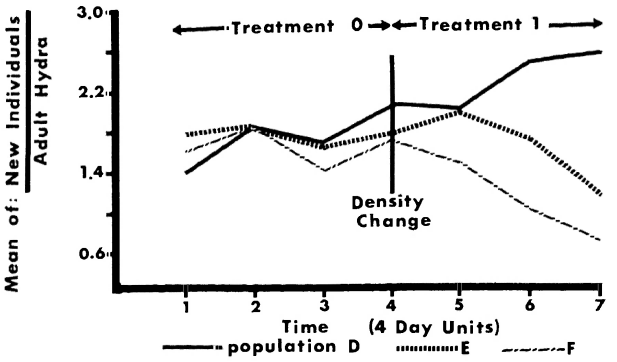


FIG. 4. Effects of abrupt decreases in crowding on growth rates in green hydras. All populations in treatment 0 are at densities of 160 hydras per dish. In treatment 1, populations D, E, and F are at densities of 20, 80, and 160 hydras per dish, respectively.

in this period are similar (2.43, 2.58, and 2.37) in comparison to mean growth rates for other densities (Fig. 2). Time affected each of the three populations in a similar manner (Time*Pop.; $F$-test: $P > 0.05$).

In the density-change period (treatment 1; Fig. 3), the densities of populations B and C were increased while population A was left uncrowded. The slopes of the plotted growth rates for these populations are significantly different ($F$-test: $P < 0.003$). Additionally, time affects each population during this period in a different manner (Time*Pop.; $F$-test: $P < 0.001$).

Mean growth rates for populations A, B, and C were calculated for each time unit in treatment 1. We compared averages of these means for each population using the Duncan Multiple Range test. Although the slopes of the lines A, B, and C are significantly different ($P < 0.05$; based on 79 observations), the Duncan Multiple Range test (based on 12 values of mean growth rates) indicates no significant differences ($P > 0.05$) between means of populations. This discrepancy could have occurred because the population lines diverged from closely spaced points. Had the experiment been longer, the effect of the original points of divergence on the means of the lines would have been less, and therefore the multiple range test probably would have indicated significant differences.

*Effects of rapidly decreased population densities.*—Effects of rapid decreases in crowding are shown in Fig. 4. Populations D, E, and F were analyzed similarly to populations A, B, and C, but were more consistent with expected results. Populations D, E, and F were not significantly different during treatment 0 (all replicates at a density of 160 hydras per dish). Likewise, the effects of time on growth rates were not significantly different (Time*Pop., $F$-test: $P > 0.05$).

After undergoing changes in density (treatment 1), the population lines diverged. Both the slopes of the lines and the time population interactions were significantly different ($F$-test: $P < 0.0001$). The Duncan Multiple range test of populations D, E, and F indicates that the control population is significantly different from the population undergoing the greatest decrease in crowding ($P < 0.01$). Results of the least significant difference test, which analyzes the means of the populations, were similar to those of the Duncan Multiple Range test.

### *Effects of crowding in hydra on endosymbionts*

As noted earlier, cultures of hydras were withdrawn at specific intervals for analysis of their endosymbionts. No correlations were evident between stresses of increased crowding of hydras and quantities of algal cells per packed volume of hydras. Likewise, no correlations in numbers of endosymbionts over time were detected.

Senescent populations and those under stress tend to have higher carotenoid to chlorophyll *a* pigment ratios, possibly indicating changes in primary productivity (Margalef 1968). Despite the large variations in amounts of chlorophyll *a* recorded for Experiments I and II, no significant differences ($\chi^2$: $P > 0.90$) in pigment ratios were detected.

### *Investigation of water-borne inhibitors*

Since no evidence was obtained that algal numbers and pigment ratios were responsible for the variable growth rates in response to crowding, the possibility of a water-borne inhibitor was examined. Regression analyses of the slopes of the population lines in treatment 1 (Fig. 5) indicate highly significant differences between populations and in time-population

medium had a deleterious effect on regeneration and growth in hydra. He indicated that the inhibition of asexual reproduction was not due to changes in quantities of ammonia, in bacterial concentration, in $CO_2$ and $O_2$ partial pressures, or to changes in pH, but was probably due to a protein inhibitor (which he attempted to identify). Unfortunately, his results were based on experiments lasting only 1 wk (an insufficient period as it may take approximately one week for hydras to acclimate to environmental changes).

To substantiate the hypothesis that a water-borne inhibitor depresses asexual reproduction, we acclimated animals first to crowded conditions (160 hydras per dish), and then to uncrowded conditions (20 hydras) and cultured in either FCM, UCCM, HCCM, or CCM. The mean growth rates of hydras in UCCM and HCCM were similar since small amounts of inhibitor were probably in UCCM while the inhibitor in HCCM would be destroyed by boiling (if it were a protein). However, neither population was significantly different from populations in CCM. Apparently there were sufficient inhibitors in the medium of all three populations to inhibit reproduction. Growth rates of hydras in CCM were consistently lower than rates for populations in either UCCM or HCCM.

The growth rates of green hydras, like those of many other aquatic organisms, are inversely related to population densities and are also affected by abrupt changes in population densities. The depressed growth rates of hydras reared in crowded conditions seems to be caused by water-borne inhibitors rather than by quantitative changes of endosymbionts and/or by their pigment ratios. This latter supposition is speculative but is supported by the data at hand and provides a useful hypothesis for future investigations.

## Acknowledgments

We thank Leon E. Gary, Jr., and J. Scott Ziesenis for aid in the statistical analyses. This study was partially supported by the National Science Foundation Grant 28951 to the North Carolina State University Phytotron.

## Literature Cited

Brown, M. E. 1946. The growth of brown trout (*Salmo trutta* Linn.). 1. Factors influencing the growth of trout fry. J. Exp. Biol. **22**:114–118.

Cook, C. B. 1972. Benefit to symbiotic zoochlorellae from feeding by green hydra. Biol. Bull. **142**:236–242.

Davis, L. V. 1966. Inhibition of growth and regeneration in hydra by crowded culture water. Nature **212**:1215–1217.

Frank, P. W., C. D. Boll, and R. W. Kelly. 1957. Vital statistics of laboratory cultures of *Daphnia pulex* DeGeer as related to density. Physiol. Zool. **30**: 287–305.

Griffing, T. 1965. Dynamics and energetics of populations of brown hydra. Ph.D. Thesis. Univ. Michigan, Ann Arbor.

Lomnicki, A., and L. B. Slobodkin. 1966. Floating in *Hydra littoralis*. Ecology **47**:881–889.

Loomis, W. F. 1954. Environmental factors controlling growth in hydra. J. Exp. Zool. **126**:223–234.

———. 1956. Induction of sexual differentiation in hydra by a volatile chemical or gas. Federation Proc. **15**:990.

Loomis, W. F., and H. M. Lenhoff. 1956. Growth and sexual differentiation of hydra in mass culture. J. Exp. Zool. **132**:555–568.

Lytle, C. F., L. G. Epp, and G. T. Barthalmus. 1971. Effects of light and other factors on host-symbiont interactions in green hydra, p. 249–264. In T. C. Cheng [ed.] Aspects of the biology of symbiosis. Univ. Park Press, Baltimore.

Margalef, R. 1968. Perspectives in ecological theory. Univ. Chicago Press, Chicago. 111 p.

Muscatine, L. 1961. Symbiosis in marine and freshwater coelenterates, p. 255–268. *In* H. M. Lenhoff and W. F. Loomis [ed.] The biology of hydra. Univ. Miami Press, Miami.

———. 1965. Symbiosis of hydra and algae—III. Extracellular products of the algae. Comp. Biochem. Physiol. **16**:77–92.

Muscatine, L., and H. M. Lenhoff. 1965. Symbiosis of hydra and algae. II. Effects of limited food and starvation on growth of symbiotic and aposymbiotic hydra. Biol. Bull. **129**:316–328.

Oschman, J. L. 1967. Structure and reproduction of the algal symbionts of *Hydra viridis*. J. Phycol. **3**: 221–228.

Park, H. D., and A. B. Ortmeyer. 1972. Growth and differentiation in hydra. II. The effect of temperature on budding in *Hydra littoralis*. J. Exp. Zool. **179**: 283–288.

Park, H. D., C. Mecca, and A. Ortmeyer. 1961. Sexual differentiation in hydra in relation to population density. Nature **191**:92–93.

Rose, S. M. 1959*a*. Population control in guppies. Am. Midl. Nat. **62**:474–481.

———. 1959*b*. Failure of survival of slowly growing members of a population. Science **129**:1026.

Smith, D., L. Muscatine, and D. Lewis. 1969. Carbohydrate movement from autotrophs to heterotrophs in parasitic and mutualistic symbiosis. Biol. Rev. **44**: 17–90.

Strickland, J. D. H., and T. R. Parsons. 1968. Spectrophotometric determination of chlorophylls and total carotenoids, p. 185–196. *In* A practical handbook of seawater analysis. Bulletin 167. Fish. Res. Bd. Can., Ottawa.