Microbial Ecology 4:207–214 (1978)

MICROBIAL ECOLOGY

Growth Responses of Ciliate Protozoa to the Abundance of Their Bacterial Prey

William D. Taylor*

Department of Zoology, University of Toronto, Toronto, Ontario M5S 1A1

Abstract. The growth rate or numerical response of five species of bactivorous ciliates to the abundance of *Enterobacter aerogenes* was examined in monoxenic culture. The ciliates *Colpidium campylum, C. colpoda, Glaucoma scintillans, G. frontata,* and *Cyclidium glaucoma* were isolated from a small pond. Four were grown in shaken cultures, while three were grown in cultures in which the bacteria were allowed to settle on the bottom of the culture vessel. Of the seven response curves generated, four had distinct thresholds, so that the Michaelis-Menten model usually fitted to ciliate numerical response curves was not appropriate. In shaken cultures, half-saturation prey densities ranged from 5.5×10^6 to 42.9×10^6 bacteria/ml. In unshaken cultures, half-saturation densities ranged from 0.057×10^6 to 14.6×10^6 bacteria/cm^2. Two species grown on both suspended and settled bacteria attained higher growth rates and had lower half-saturation prey densities feeding on settled bacteria.

Introduction

There has been a recent increase in interest in the relationship between bacteria in aquatic ecosystems and the ciliate protozoans which graze on them. Much of this interest has arisen from the increasing urgency of understanding the effects of organic enrichment on aquatic ecosystems, and also from the realization that heterotrophic bacteria play a major role in secondary productivity and nutrient cycling [3,23].

An aspect of the relationship between bacteria and their ciliate predators which has been the object of several studies is the growth rate or numerical response of ciliates to the density of their bacterial prey [1,2,4,6–9,12,13,16,18,-21,25,28]. Despite the considerable attention this topic has received, several critical questions remain.

Although some of the previously mentioned studies (e.g., 2,7) found that ciliates did not multiply until high bacterial densities were available to them, the more quantitative ones in which response curves were generated assumed a fit

* Present Address: Dept. of Biology, University of Waterloo, Waterloo, Ontario N2L 3G1, Canada

0095–3628/78/0004–0207 $01.60

through the origin [1,4,6,12,21,25,28]. This latter group of continuous-culture studies assumed that the relationship fit the classic Michaelis-Menten curve or the modified version proposed by Contois [5]. In view of the importance of thresholds in stabilizing prey populations [14,24], the conflicting evidence even using the same species or genera (e.g., 7 and 25, 2 and 1,12), and the general expectation that filter-feeding species should stop feeding at unprofitably low prey densities, more investigation is needed.

A second problem is the extent to which ciliate species differ in the prey densities over which they show a changing response. Although several ciliate species have now been investigated, individual studies have used only single species. Because of differences in experimental design, prey species used, units of measurement, temperature, and other parameters, comparisons are difficult. A comparative study is required.

A final problem is that the above studies all used a stirred bacterial suspension so that densities would be uniform. Many or most ciliates graze surfaces, feeding on nonmotile or attached prey. Even pelagic ciliates may be associated with particles [12].

It is the aim of this paper to help resolve these three problem areas by using five ciliate species, by using both suspended and settled bacteria, and by paying particular attention to severely limiting bacterial densities.

Materials and Methods

The ciliates used in this study were collected from a small, soft-water pond in central Ontario (79°29'13"W, 44°52'42"N). They were *Colpidium campylum* (Stokes), *Colpidium colpoda* (Ehrenberg), *Glaucoma scintillans* (Ehrenberg), *Glaucoma frontata* (Stokes), and *Cyclidium glaucoma* (Müller). The prey bacterium, *Enterobacter* (=*Aerobacter*) *aerogenes*, was obtained from the culture collection of the School of Hygiene, University of Toronto. Details of isolation and routine maintenance of stocks have been previously published [26,27].

Two experimental designs were used; one to examine numerical response to suspended bacteria and the other to examine numerical response to settled bacteria. The former were carried out in 20 × 150 mm screw-capped test tubes containing 10 ml of medium which were rotated at 10 rpm in a horizontal position with a rotary shaker. The latter were carried out in 5 cm Petri dishes containing 4 ml of medium.

The culture medium for both types of experiments was a 0.15% w/v pond-water extract of cerophyl (Cerophyl Laboratories, Inc., Kansas City, Mo.). This medium was filtered (0.45 μm) and autoclaved before use. Cerophyl, a powder made from cereal grasses, supports the growth of bacteria but is not by itself a food source for ciliates. To prepare various prey densities, *Enterobacter* was grown for 48 hours at 25°C. This treatment ensured that the cells were in stationary phase. The resultant bacterial concentration was then determined with the aid of a Petroff-Hauser bacteria-counting chamber, and the desired prey densities were prepared by dilution with membrane-filtered pond water. In cases where densities in excess of 10^8/ml were required, the bacteria were allowed to settle and some of the supernatant was removed.

At the start of each growth rate determination a known number of ciliates (in the range of 5 to 20) were added to each test tube or Petri dish. After 24 hours' incubation at 20°C, the resultant population was counted for the determination of population growth rate by removing the cells singly with a micropipet. Some cells were then transferred to a new culture for further determinations. Growth rates were not recorded for the first 24-hour period to allow for the adjustment of growth rate to prey density. Growth rates were estimated as $(\log_e N_t - \log_e N_0)/t$, where N_0 and N_t are the initial and final

number of ciliates and t is the elapsed time. When a negative growth rate was recorded, the experiment was repeated using cells from a higher prey density to replace those lost.

In suspension-feeding experiments the initial and final prey densities were averaged and a mean prey density recorded. The results from cultures changing more than 50% were discarded. These cases were rare. The absolute change in density was usually slight. High relative changes in prey density therefore occurred in low prey density cultures. Final prey density was not determined in the settled bacteria cultures since results from the suspended bacteria cultures indicated that the ciliates did not have a noticeable effect on bacterial numbers over the 24-hour duration of the experiments.

In experiments measuring growth response to settled bacteria, prey density was recorded as numbers/cm^2. This density was calculated by multiplying the number/ml times the culture volume (4 ml) and dividing by the area of the bottom of the Petri dish (20 cm^2).

The relationship between growth rate and prey density was fitted to a modified Michaelis-Menten hyperbolic function of the form $y = r_m(x-t)/[(K_s-t) + (x-t)]$, where the ordinate (y) is the specific growth rate, the abscissa (x) is the prey density, r_m is the maximum growth rate, K_s is the half-saturation value (i.e., the prey density which allows a growth rate of $r_m/2$), and t is the threshold prey density (the highest value of x at which y is zero).

The threshold prey density was estimated by inspection while K_s and r_m were determined by the reciprocal plot method [10,11]. This method uses the least squares method to fit the line $(x-t) = r_m[(x-t)/y] - (K_s-t)$. It is appropriate for constant absolute error in y.

Results

The responses of four ciliate species to the density of suspended bacteria are given in Fig. 1. *Glaucoma scintillans* (Fig. 1d) grew erratically. The line shown was fitted using the maximum growth rate observed on settled bacteria. The erratic growth of this species probably reflects that it is an obligate deposit-feeder and does not ingest prey when not in contact with a surface. Its feeding is therefore inhibited by shaking. *G. frontata* would not grow in shaken cultures.

The other three species, *C. campylum, C. colpoda,* and *C. glaucoma,* have half-saturation prey densities of 13, 5.5, and 42.9 $\times$ 10^6 bacteria/ml, respectively. Each showed a threshold in its response. The half-saturation and threshold values ranged approximately six- to seven-fold. The modified Michaelis-Menten equation appears to be a reasonable approximation of true response, although there may be a tendency for it to saturate too slowly.

The response of three species to the density of settled bacteria is given in Fig. 2. In this deposit-feeding mode, *G. scintillans* was the species most effective at using low prey densities. The Michaelis-Menten equation was a poor fit, probably due to the increased significance of experimental error at such low prey densities.

C. campylum had a K_s value of 6.29 $\times$ 10^5 bacteria/cm^2 on settled bacteria, which in units of volume is 3.15 $\times$ 10^6/ml. This indicates a considerable reduction from the suspension-feeding value of 13.0 $\times$ 10^6/ml, indicating that *C. campylum* can exploit settling of the bacteria. *C. campylum* also had a higher predicted value of r_m (0.170 versus 0.163 h^{-1}) on settled bacteria, which suggests that shaking has an adverse effect on growth.

The overall range in K_s values, from *G. scintillans* to *G. frontata,* was 5.7 $\times$ 10^4 to 1.46 $\times$ 10^7 bacteria/cm^2. *G. frontata* had a threshold in its response to the density of settled bacteria, but *C. campylum* and *G. scintillans* did not appear to

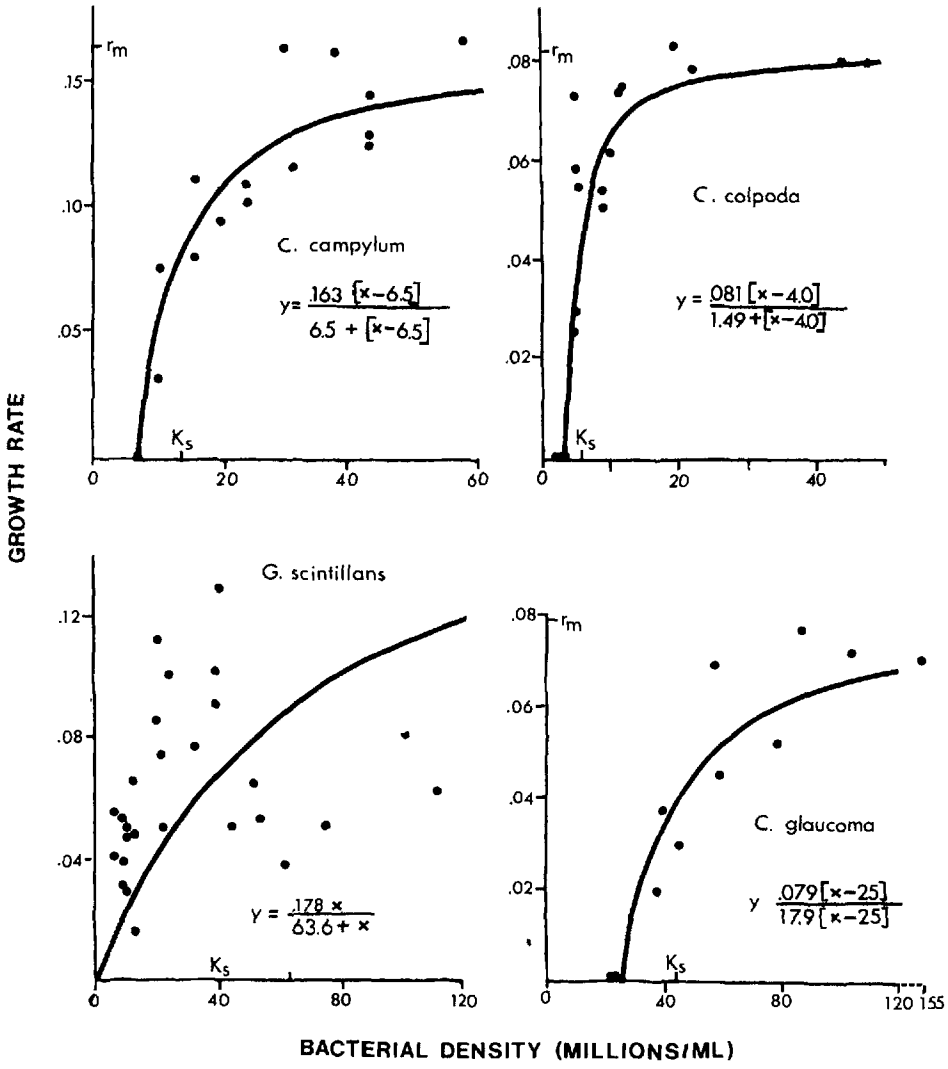

Fig. 1. The numerical responses of four species of bactivorous ciliates to the density of suspended *Aerobacter aerogenes*.

have thresholds, at least at densities high enough to be detected by this experimental design.

Discussion

The numerical response curves shown in Figs. 1 and 2 illustrate that thresholds in the response of ciliates to their bacterial prey do exist. Their apparent absence in the responses of *C. campylum* and *G. scintillans* to settled bacteria is suspect,

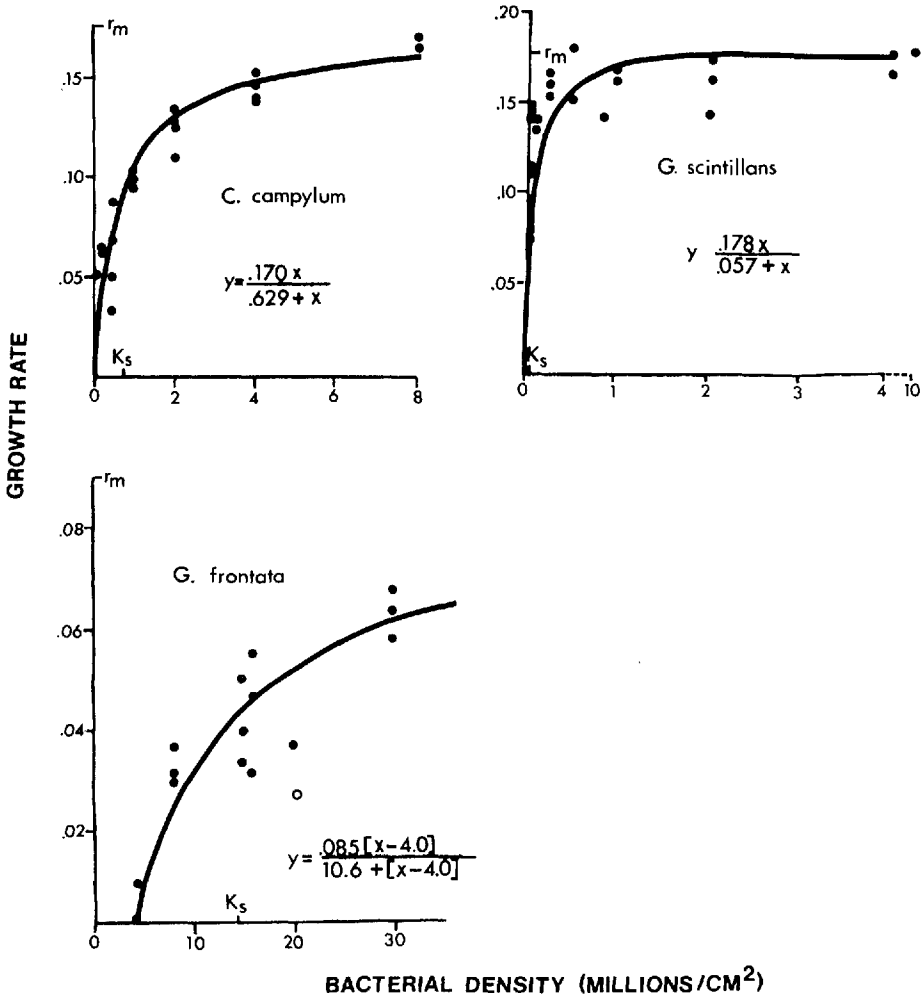

Fig. 2. The numerical responses of three species of bactivorous ciliates to the density of settled *Aerobacter aerogenes*. The hollow circle represents a point omitted from the regression analysis.

because these two cases involved the lowest prey densities. From an examination of the literature it appears that continuous culture studies do not find thresholds [1,4,6,12,21,25,28], although other studies do [2,7,18]. This may be due not only to difficulties in studying near-zero (and near-maximal) growth rates using continuous culture, but also to a tendency to assume that all continuous cultures display chemostat kinetics, despite warnings to the contrary [15]. It is likely that relationships demonstrated for organisms limited by concentrations of dissolved nutrients, such as bacteria and algae, do not necessarily apply to phagotrophic protozoans.

Jost et al. [16] found that the inadequacy of the Michaelis-Menten (or Monod) model at low bacterial densities precluded its use in realistic models of simple

microbial food chains and webs involving the ciliate *Tetrahemena* as the predator. They introduced the "multiple saturation model" [17], which features an accelerating growth rate response over low bacterial densities, resulting in a sigmoidal numerical response curve. The multiple saturation model has been recently applied to *Colpoda steini* [9].

Thresholds are an expected property of numerical response curves. There must always be a range of low prey densities over which ingestion cannot offset maintenance metabolism, so that population growth rate is less than or equal to zero. This has been demonstrated by Laybourn and Stewart [18] for *C. campylum*. But the net response of a predator population to the density of its prey depends on the nature of the combined numerical and functional (or ingestion rate) response. It is therefore important to understand whether the numerical response thresholds observed correspond to underlying functional response thresholds. If filtering the medium for bacteria represents a substantial energy cost, it would seem advantageous to limit filtering activity when prey densities are too low to provide a compensating energy return. Furthermore, in a spatially heterogeneous environment, an organism may find it more profitable to search for a higher prey density than to feed at a low one. Lehman [19] has recently presented a theoretical investigation of optimal response curves for filter-feeding organisms. The induction of phagocytosis in *Tetrahymena* has received considerable attention (see Rasmussen [22] for a review), although ecological aspects, including threshold prey densities, are an area needing further attention.

Even among the few species examined in this study, it appears that considerable interspecific variation is present with respect to the prey density required for population growth. For example, *C. colpoda* is virtually prey saturated at the threshold level for *C. glaucoma*. Taking the three suspension-feeding species together, one sees an increasing growth response to bacterial density over the range 4.0×10^6 to 1.5×10^8 bacteria/ml. There is some indication in the literature [1,12] that the marine scuticociliate *Uronema* can feed at considerably lower densities (K_s values of 1.5×10^6 *Serratia marinorubra*/ml [12] and 6.85×10^5 *Vibrio* sp./ml [1] have been reported). A third study, however, found a "critical density" or threshold of 10^6–10^7 bacteria/ml for *U. nigricans* [2].

The observed responses of the ciliate species to settled bacteria showed even more interspecific variation. Although the density of bacteria in these experiments was not exactly known, because the proportion of the bacteria which are actually settled was not known and because uniform distribution of the bacteria was not guaranteed by shaking, the experiments should provide a reliable basis for comparison. It is clear that *G. scintillans, G. frontata,* and *C. campylum* can exploit the settling of bacteria to grow at prey densities which would be too low to support growth in shaken cultures. This observation may in part explain the apparent discrepancy between the densities of bacteria required by ciliates for population growth and the observed densities of bacteria in aquatic habitats.

At the outset of this investigation it was expected that there might be a positive relationship between K_s and r_m among species. This expectation arose from the idea that K_s would be inversely related to competitive ability, at least in homogeneous environments, and that competitive ability and r_m would be negatively related [15, 20]. This was not the case; there is no indication of such a

relationship among this admittedly small sample of species. If a relationship exists at all, it is a negative one.

Acknowledgments This study is part of a thesis submitted to the University of Toronto in partial fulfillment of the requirements of the Ph.D. degree. The research was supported by a National Research Council of Canada operating grant to Prof. J. Berger, Dept. of Zoology, University of Toronto. Prof. Berger also suggested several improvements in the manuscript. Financial assistance from an Ontario Graduate Scholarship is gratefully acknowledged.

References

1. Ashby, R. E.: Long term variations in a protozoan chemostat culture. J. Exp. Mar. Biol. Ecol. **24,** 227–235 (1976)
2. Berk, S. G., R. R. Colwell, and E. B. Small: A study of feeding responses to bacterial prey by estuarine ciliates. Trans. Am. Microsc. Soc. **95,** 514–520 (1976)
3. Bott, T. L.: Nutrient cycles in natural systems: Microbial involvement. In: J. Tourbier and R. W. Pierson (Eds.): Biological Control of Water Pollution, pp. 41–42. University of Pennsylvania Press, Philadelphia (1976)
4. Canale, R. P., T. D. Lustig, P. M. Kehrberger, and J. E. Salvo: Experimental and mathematical modelling studies of protozoan predation on bacteria. Biotechnol. Bioeng. **15,** 707–728 (1973)
5. Contois, D. E.: Kinetics of bacterial growth: relationship between population density and specific growth rate of continuous cultures. J. Gen. Microbiol. **21,** 40–50 (1959)
6. Curds, C. R., and A. Cockburn: Continuous monoxenic culture of *Tetrahymena pyriformis*. J. Gen. Microbiol. **66,** 95–108 (1971)
7. Dive, D.: Influence de la concentration bactérienne sur la croissance de *Colpidium campylum*. J. Protozool. **22,** 545–550 (1975)
8. Drake, J. F., and H. M. Tsuchiya: Predation on *Escherichia coli* by *Colpoda steini*. Appl. Environ. Microbiol. **31,** 870–874 (1976)
9. Drake, J. F., and H. M. Tsuchiya: Growth kinetics of *Colpoda steini* on *Escherichia coli*. Appl. Environ. Microbiol. **34,** 18–22 (1977)
10. Endrenyi, L.: Statistical problems of kinetic model building. Symp. Biol. Hung. **18,** 11–30 (1974)
11. Endrenyi, L., and F. H. F. Kwong: Design and analysis of hyperbolic kinetic and binding experiments. In: H. C. Hemker and B. Hess (Eds.): Analysis and Simulation of Biochemical Systems, pp. 219–237. North-Holland, Amsterdam (1972)
12. Hamilton, R. D., and J. E. Preslan: Observations on the continuous culture of a planktonic phagotrophic protozoan. J. Exp. Mar. Biol. Ecol. **5,** 94–104 (1970)
13. Harding, J. P.: Quantitative studies on the ciliate *Glaucoma*. I. The regulation of the size and fission rate by the bacterial food supply. J. Exp. Biol. **14,** 422–430 (1937)
14. Holling, C. S.: The functional response of predators to prey density and its role in mimicry and population regulation. Mem. Entomol. Soc. Can. **45,** 1–60 (1965)
15. Jannasch, H. W.: Steady state and the chemostat in ecology. Limnol. Oceanogr. **10,** 716–720 (1974)
16. Jost, J. L., J. F. Drake, A. G. Fredrickson, and H. M. Tsuchiya: Interactions of *Tetrahymena pyriformis, Escherichia coli, Azotobacter vinelandii,* and glucose in a minimal medium. J. Bacteriol. **113,** 834–840 (1973)
17. Jost, J. L., J. F. Drake, H. M. Tsuchiya, and A. G. Fredrickson: Microbial food chains and webs. J. Theor. Biol. **41,** 461–484 (1973)
18. Laybourn, J. E. M., and J. M. Stewart: Studies on consumption and growth in the ciliate *Colpidium campylum* Stokes. J. Anim. Ecol. **44,** 165–174 (1975)
19. Lehman, J. T.: The filter feeder as an optimal forager, and the predicted shapes of feeding curves. Limnol. Oceanogr. **21,** 501–516 (1976)
20. Pianka, E. R.: On r- and K-selection. Am. Nat. **104,** 592–597 (1970)

21. Proper, G., and J. C. Garver: Mass culture of the protozoa *Colpoda steini*. Biotechnol. Bioeng. **8,** 287–296 (1966)
22. Rasmussen, L.: Nutrient uptake in *Tetrahymena pyriformis*. Carlsberg Res. Commun. **41,** 143–167 (1976)
23. Sieburth, J. M.: Bacterial substrates and productivity in marine ecosystems. Annu. Rev. Ecol. Syst. **7,** 259–285 (1976)
24. Steele, J. H.: The Structure of Marine Ecosystems. Harvard University Press, Cambridge, Mass. (1974)
25. Sudo, R., K. Kobayashi, and S. Aiba: Some experiments and analysis of a predator-prey model: interaction between *Colpidium campylum* and *Alcaligenes faecalis* in continuous and mixed culture. Biotechnol. Bioeng. **17,** 167–184 (1975)
26. Taylor, W. D., and J. Berger: Growth of *Colpidium campylum* in monoxenic batch culture. Can. J. Zool. **54,** 392–398 (1976)
27. Taylor, W. D., and J. Berger: Growth responses of cohabiting ciliate protozoa to various prey bacteria. Can. J. Zool. **54,** 1111–1114 (1976)
28. Villarreal, E., R. R. Canale, and Z. Akcasu: Transport equations for a microbial predator-prey community. Microb. Ecol. **3,** 131–142 (1977)