Summer 1968

Colonial independence of feeding among Zooids of the Ectoproct Lophodella carteri (Hyatt)

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by

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A thesis submitted in partial fulfillment of the requirements for the degree of Master of Science at the University of Richmond.

August, 1968

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Abstract

Possible interactions of feeding between zooids of colonies of the freshwater ectoproct *Lophopodella carteri* were investigated. Suspensions of *Euglena gracilis* at a concentration of 558×10^3 cells ml^{-1} were cleared by *L. carteri* at a rate of 2.7×10^{-3} ml min^{-1} $zooid^{-1}$ for 15 minute periods. The rate of ingestion of *E. gracilis* by *L. carteri* was independent of the size of colony and decreased exponentially with time, declining to about one third the original rate in 60 minutes.

Acknowledgements

Sincere thanks are expressed to the following for their help during the study. Drs. W. S. Woolcott, W. R. Tenney and F. B. Leftwich of the Biology Department served as members of my advisory committee. Dr. A. E. Campbell of the Physics Department pointed out the problems associated with the use of a radioisotope. Mr. T. B. Vassar of the Mathematics Department offered mathematical assistance. Dr. J. S. Bullivant of the New Zealand Oceanographic Institute kindly supplied pertinent unpublished data. Special thanks are due Dr. J. W. Bishop of the Biology Department without whose help and encouragement, both as mentor and friend, I could not have finished; and to my wife, Susan, who listened patiently.

Introduction

It is well recognized that intraspecific interactions may result from the aggregation of organisms, e.g. Allee's Principle, that undercrowding as well as overcrowding limits a population. Interactions of feeding represent one parameter which influences a population.

The effect of clumping on responses of feeding among animals has most often been measured as a function of density of prey rather than density of predator (Gibb, 1962; Holling, 1965; Huffaker, 1958). Infrequent references to interactions of feeding due to aggregations of predators occur in the literature, but they usually describe motile animals. Ivlev (1961) reported that rates of feeding of carp and goldfish decrease with density, but those of bullhead catfish increase as the number of individuals increases to an optimum density. He compared the latter interaction with the tendency of terrestrial predators to increase their rate of feeding in the presence of competitors.

Although information on feeding of aquatic invertebrates is abundant, and suspension feeders have been extensively studied (Jørgensen, 1966a); interactions of feeding due to clumping among sessile animals have not been described. One beneficial effect is suggested by the study on sponges by Bidder (1923) who found that the volume of water pumped is greater for aggregations of choanoflagellates than for the sum of individual

collar cells.

It is logical that disadvantages can also result from aggregations of sessile suspension feeders if water currents produced by adjoining individuals result in turbulence, rather than confluence.

Either advantageous or detrimental interactions resulting from clumping should be revealed by determining the relationship between the rate of food uptake and the density of animals. The objective of this study is to investigate this relationship in the freshwater ectoproct Lophopodella carteri, which was selected for its highly aggregated colonial form and its availability.

Methods

Colonies of L. carteri were collected at the Virginia State Fish Cultural Station in Stevensville, King and Queen County, Virginia, during the summer of 1967. They were held in glass containers in spring water, without food, one to eleven days prior to experiments. The ambient water temperature ranged from 23 to 26 C. Some individual zooids used for visual observations were obtained from the germination of statoblasts produced by animals held in the laboratory.

Euglena gracilis was used for food because of its ease of culture and motility. The latter property eliminated the necessity of constant stirring. An agnotobiotic, unialgal culture of E. gracilis was grown in a suspension of boiled wheat extract and powdered skim milk in spring water. After it attained a maximum concentration of approximately 2×10^6 cells ml^{-1},

15 μC of carbon-14, in the form of $NaHC^{14}O_3$ was added to the culture. Three days incubation was allowed following the addition of the radioisotope before the E. gracilis were used in a feeding experiment. Subsamples of the algal culture were filtered through cotton gauze to remove clumps of bacteria and detritus before each experiment.

The direction and speed of the feeding currents produced by individual zooids were observed directly and were photographed with a motion picture camera equipped with closeup lenses. The time required for food to pass through the gut of L. carteri was determined for individual zooids feeding on E. gracilis.

Subsamples of algae (5 or 10 ml) were introduced into each of several vials that had inside diameters of 18 or 22 mm (diameter had no statistically significant effect on rate of ingestion at the 5% level). Range of concentration of E. gracilis was 175×10^3 to 700×10^3 cells ml^{-1} (concentration of food had no statistically significant effect with a 15 minute feeding time at the 5% level, but was significant with a 60 minute feeding time: $r=0.64$). A single colony of known number of zooids was placed into each of the vials, which were shaken briefly to ensure uniform dispersion of food. Three vials containing algae without L. carteri were used as controls during each experiment.

At the end of the feeding time, the vials were shaken and a 1.0 or 2.0 ml aliquot was withdrawn. The sample was filtered onto 0.3 μ pore size millipore paper. Each filter paper disc with its sample of algae was placed into a 2.54 cm diameter low-walled planchet with a small amount

of saliva as an adhesive to prevent the edges of the paper from curling. The detectable radioactivity of each sample was determined, using a Geiger-Muller gas flow counter equipped with a thin window (100 μg cm^{-2}) and scaler. All counts (sample and background) were made for 10 minutes at 1500 volts. The average coefficient of variation of the counts was estimated at 1.6%. The control vials were sampled at the end of the experiment in the same manner as the experimentals; and in addition, two 0.1 ml aliquots were removed from each control and placed into a Palmer counting cell for direct density determinations. These were made with the aid of a compound microscope with an ocular grid, at a magnification of 100. Ten fields were counted from each aliquot, and the mean number of cells $field^{-1}$ was multiplied by a calculated conversion factor of 11080.3, resulting in cells ml^{-1}. Differences in radioactivity between each experimental sample and the mean of the three control samples were considered equivalent to the activity of the cells ingested by L. carteri.

Rates of ingestion (cells eaten min^{-1} $zooid^{-1}$) were estimated using the following equation:

$$I.R. = \frac{(C.R.)\ (vol.)(k)}{(\#zooids)\ (k)\ (t)}$$

where C.R. is the difference between the control and experimental count rates ml^{-1} of sample (corrected for background), vol. is the volume of algal suspension in which the L. carteri colony has been feeding, #zooids is the colony size, k is the number of E. gracilis required to produce one count min^{-1}, and t is the feeding time in minutes.

Results

The feeding currents of L. carteri, propelled by two tracts of lateral cilia on each tentacle (Atkins, 1932) approached the lophophore frontally, or perpendicular to the plane described by the arms of the lophophore (Figs. 1 and 2). The frontal cilia on each tentacle beat effectively downward, and directed food caught in the basket, formed by the tentacles, toward troughs between the rows of tentacles on the arms of the lophophore. The troughs funnelled food toward the mouth, located at the junction of the two arms within the area enclosed by the tentacles. The average minimum distance from the tentacular crown (frontal side) over which water was drawn into the lophophore was 5 mm. Some of the water, after passing between the tentacles, formed eddy currents of about 2 mm diameter on all sides of the tentacular crown, and thus was drawn back around and filtered again. Because the afferent stream was disturbed as it passed through the tentacular crown, the efferent stream appeared to be limited to about 4 mm beyond the abfrontal side of the lophophore.

The average velocity of the afferent stream directly in front of the lophophore was determined as 0.55 mm sec^{-1} by timing food particles over the 5 mm distance from the point at which they first became caught in the feeding current. The determinations of current velocity were checked with the aid of motion picture filming of individual zooids, using a yeast suspension to delineate the currents.

The feeding current appeared to continue unabated for at least two hours, even in the presence of abundant food, but the actual velocity was

not determined at the end of this time. Food collected in clumps in the pharynx and was swallowed by a peristaltic-like movement about every 30 seconds. Excess food built up around the epistome (flap of tissue over mouth) during feeding and periodically, at intervals of about one minute, spilled over between the tentacles. At an undetermined high concentration of E. gracilis, about two hours was required for the production of the first fecal pellets containing this food.

The mean rate of ingestion for 15 minute experiments was 1475 cells min^{-1} $zooid^{-1}$, and for 60 minute experiments was 818 cells min^{-1} $zooid^{-1}$ (Table 1). The rate of ingestion $zooid^{-1}$ was independent of the size of the colony. The coefficient of correlation for rate of ingestion $zooid^{-1}$ vs. size of colony was not significant at the 5% level, either for the 15 minute or 60 minute experiments. In order to ascertain if the poor correlation of rate of ingestion vs. size of colony was due to L. carteri becoming satiated before the feeding time had ended, rate of ingestion vs. time was tested. The results showed that the rate of ingestion decreased approximately exponentially with time (Fig. 3). The regression equation describing this relationship is:

$$Y = 3.53 - 0.0089X$$

where $Y=\log_{10}$I.R. and X=time in minutes.

These results indicated that the animals were still feeding at a high rate at the end of the 15 minute feeding time. After 60 minutes they fed at about one third the original rate.

Discussion

The only previous study emphasizing quantitative aspects of feeding by ectoprocts concerns the gymnolaemate (marine) ectoproct, Zoobotryon verticillatum (Bullivant, 1967). The individual zooids of this species resemble those of L. carteri; however the colonies differ greatly, as Z. verticillatum is characterized by branching, diffuse colonies that contrast with the compact, uniform colonies of L. carteri.

The average rates of ingestion of L. carteri are approximately seven times higher than those of Z. verticillatum (Table 2). The difference is even more striking if the volume of individual cells is taken into account, as the four species of algae used for food in the Z. verticillatum study are all minute compared to E. gracilis (Table 2). The results of experiments with a euglenoid flagellate (Eutreptia marina) used in the study on Z. verticillatum are inconclusive. Some of the disparity in rates of ingestion between the two ectoprocts may be explained by size difference, as the dry weight zooid^{-1} of L. carteri is 0.04 mg, almost four times the weight of Z. verticillatum zooids (0.0109 mg). Also, the data for L. carteri represent short feeding times preceded by starvation, whereas the rates of ingestion of Z. verticillatum were determined from extended feeding intervals following conditioning in equivalent concentrations of food.

Rates of clearance of water are commonly used as indices of feeding for suspension feeders (Gauld, 1951; Ryther, 1954) and for the present study the rate of clearance is assumed equal to the rate of ingestion divided by the initial concentration of food. The rates of clearance between the two

species are much closer than the rates of ingestion, with Z. verticillatum slightly exceeding L. carteri (Table 2).

There are at least two possible explanations of the inability to detect interactions of feeding of zooids in the present study. The first hypothesis is that no interaction of feeding occurs, despite the proximity of the intracolonial zooids. Perhaps each zooid in a colony turns its lophophore toward the least disturbed water and thereby reduces the effect of neighboring currents. Another explanation is that interactions of feeding are masked by effects on rate of ingestion of factors other than rate of procurement of food.

If the concentrations of E. gracilis used for the experiments are so high that the ability of L. carteri to take up food or to pass it through the gut becomes limiting, then enhanced feeding currents will not result in increased ingestion. The possibility that this occurs follows the concept of a satiation concentration of food, described by Bullivant (1967). This concept proposes that a suspension feeder ingests food at a maximum rate that will be unaffected by further increase in food concentration. This results in uniform rates of ingestion among all members of all colonies, and enhanced or decreased ability to pump is obscured. Since the concentrations of food in this study are considerably higher than those in Bullivant's work (Table 2), and the rate of ingestion is not correlated with concentration of food, there is a possibility that the satiation concentration for L. carteri has been exceeded.

The periodic overflow of "superfluous" food in the mouth region of

L. carteri indicates that food is delivered to the mouth faster than it is ingested, which suggests that the satiation concentration has been exceeded. The results of the 15 minute experiments also indicate that this is true as no significant increase of rate of ingestion follows concentration increase. The increase of rate of ingestion with concentration in the 60 minute runs remains unexplained, although the range of concentration is considerably greater in these experiments than in the 15 minute runs.

The decrease of rate of ingestion with time is considered evidence that feeding in L. carteri decreases as the gut fills up. The regulating mechanism, which apparently acts to prevent overfeeding, is not clear. The epistome is a possible ingestion-regulating structure in phylactolaemates. This highly sensitive structure can change the size of the mouth opening, although it cannot completely close it (Hyman, 1959). The epistome overlies the nervous center of the zooid, located between the mouth and the anus, and it is thought to act in a chemoreceptive capacity for rejection of unwanted food (Hyman, 1959). Gymnolaemates do not possess epistomes, and the only gymnolaemate in which feeding has been measured, Z. verticillatum, shows no change of rate of ingestion with time (Bullivant, 1967).

Another possible regulator of ingestion of L. carteri would be a variable feeding current. As described in the present study, the current appears to remain undiminished, but some authors claim that the cilia of phylactolaemates do not beat constantly (Hyman, 1959).

Production of fecal pellets by L. carteri appears to be independent of ingestion, and could possibly limit the uptake of food. As described above,

the first fecal pellets containing E. gracilis are produced about two hours after the animals are placed into a food suspension. This time compares favorably with animals held in lake water practically devoid of food in which about 10 empty fecal pellets zooid^{-1} are produced in 20 hours. The minimum time required to synthesize a fecal pellet might limit the rate at which the gut can be emptied of undigested food, and thus limit the rate at which ingestion can occur.

Another possible explanation for the decrease in rate of ingestion with time would follow if E. gracilis were shown to initiate a negative response in L. carteri. According to Rogick (1937), Euglena is unfavorable as food for L. carteri. Fecal pellets containing intact, living E. gracilis were found in the present study, but this is thought to indicate superfluous feeding, rather than non-assimilation. Radioactivity determination of a single colony of L. carteri fed tagged E. gracilis for 24 hours and then isolated in fresh-water for 36 hours showed an activity of 21.9 counts min^{-1} above background, which indicated that the ectoproct assimilates E. gracilis.

To conclude, the original hypothesis, that interactions of feeding would result from clumping in the ectoproct L. carteri, was not supported by data. Further work is needed to ascertain if an interaction resulting from enhanced or diminished feeding currents exists at concentrations of food more in line with natural densities of algae. This will involve a problem often encountered in experimental trophic ecology (Jørgensen, 1966b), namely the measurement of small changes in concentration of suspension organisms at densities representative of natural populations.

TABLE 1

Ingestion Rate of *E. gracilis* by *L. carteri*

Feeding time (min)	N	Mean colony size ± S.D. (no. zooids)	Mean algal conc. ± S.D. (cells ml^{-1})	Mean holding time of animals (days)	Mean temp. (C)	Mean rate of ingestion (cells min^{-1} $zooid^{-1}$) ± S.E.
60	45	18.3 ± 16.8	368,000 ± 192,000	5.6	25.7	818 ± 78
15	63	33.5 ± 16.4	558,000 ± 40,000	2.0	23.6	1475 ± 81

TABLE 2

Comparison of Ingestion Rates and Clearance Rates of L. carteri and Z. verticillatum[1]

	L. carteri	Z. verticillatum
Dry weight zooid^{-1} (mg)	0.04	0.0109
Ingestion rate (cells min^{-1} zooid^{-1})	E. gracilis - 1475	Monochrysis sp. - 47 Phaodactylum sp. - 58 Dunaliella sp. - 21 Cricosphaera sp. - 25
Ingestion rate (mm^3 min^{-1} zooid^{-1})	E. gracilis - 6.174×10^{-3}	Monochrysis sp. - 0.004×10^{-3} Phaodactylum sp. - 0.006×10^{-3} Dunaliella sp. - 0.005×10^{-3} Cricosphaera sp. - 0.02×10^{-3}
Mean clearance rate (ml min^{-1} zooid^{-1})	food conc. 0.0027 - E. gracilis - (558,000 cells ml^{-1})	food conc. 0.006 - Monochrysis sp. - (4,504 cells ml^{-1}) 0.009 - Phaodactylum sp. - (5,024 cells ml^{-1}) 0.003 - Dunaliella sp. - (4,386 cells ml^{-1}) 0.012 - Cricosphaera sp. - (936 cells ml^{-1})
Mean food volume (μ^3)	E. gracilis - 4186	Monochrysis sp. - 81 Phaodactylum sp. - 103 Dunaliella sp. - 257 Cricosphaera sp. - 796

[1] All numbers under Z. verticillatum are approximations from Bullivant (1967).

Figure 1

Views of single zooid of L. carteri showing ciliary feeding currents.

A. Latero-frontal view.

B. Latero-abfrontal view.

Arrows are colored on one surface only to give three-dimensional effect. Dark surfaces of arrows are convex; light surfaces are concave.

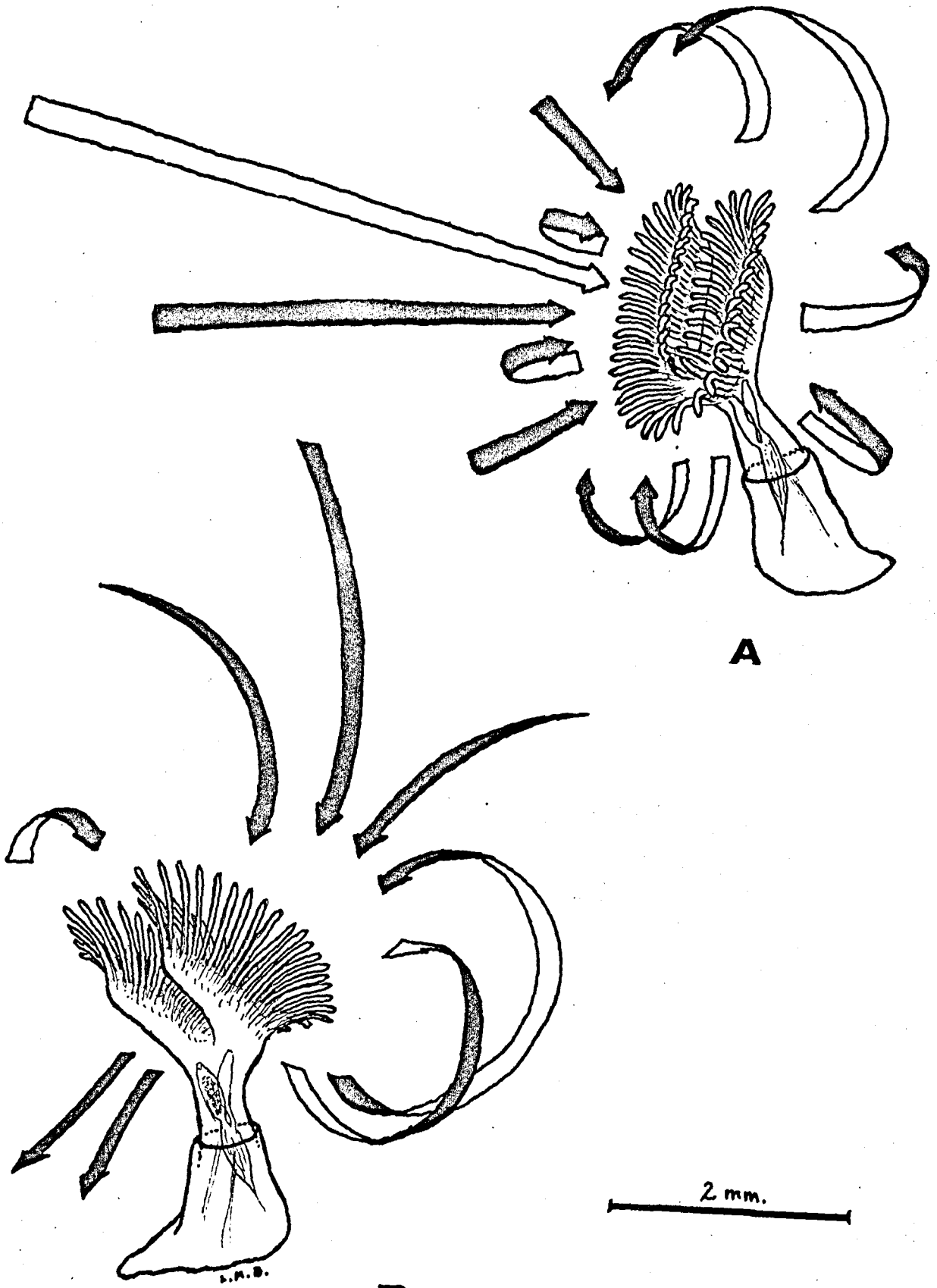

A
2 mm.
B

Figure 2

Views of single zooid of L. carteri showing ciliary feeding currents.

A. Frontal view.

B. Dorso-abfrontal view.

Arrows are colored on one surface only to give three-dimensional effect. Dark surfaces of arrows are convex; light surfaces are concave.

Key:

m - mouth; tr - troughs on arms of lophophore

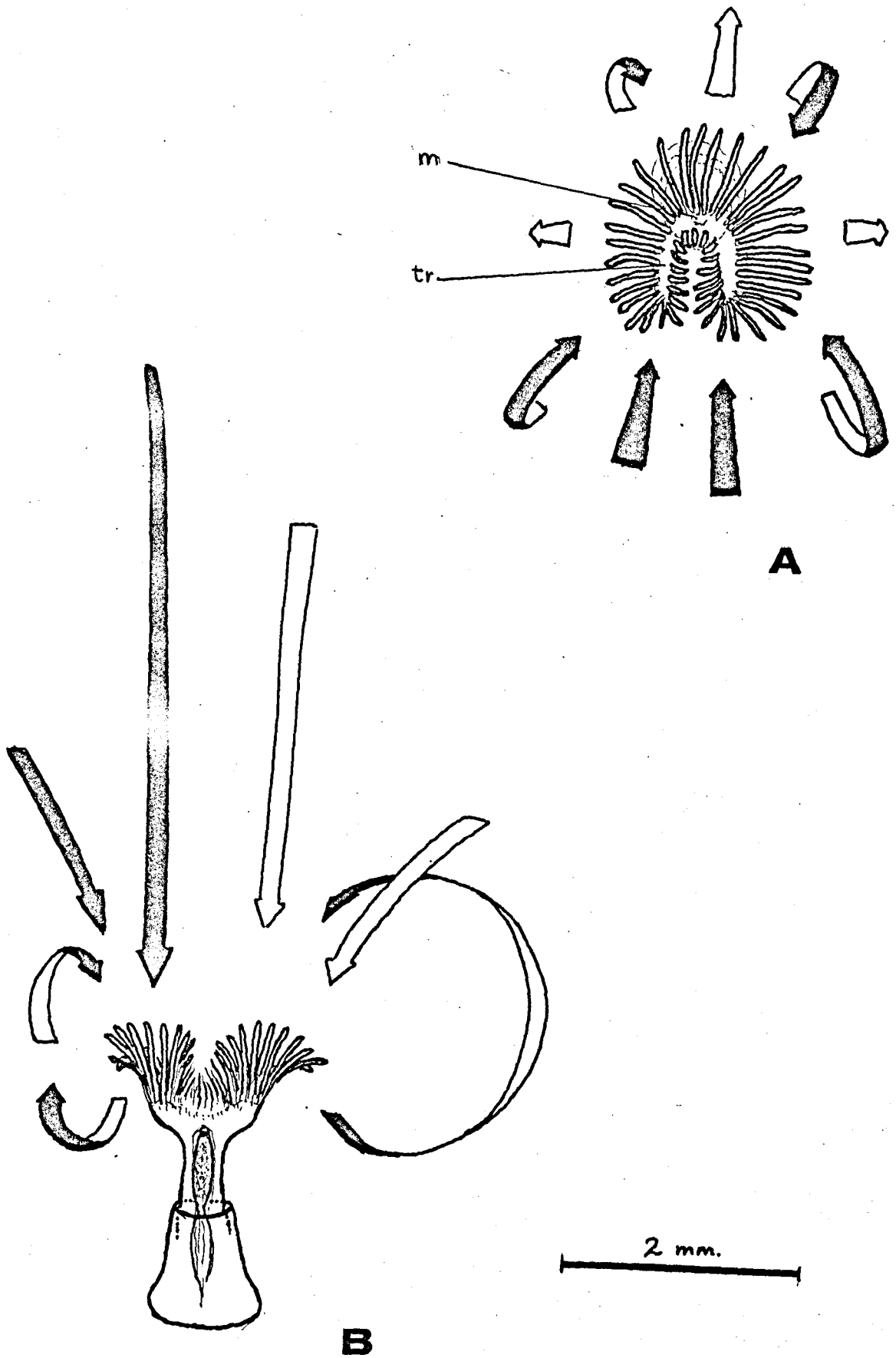
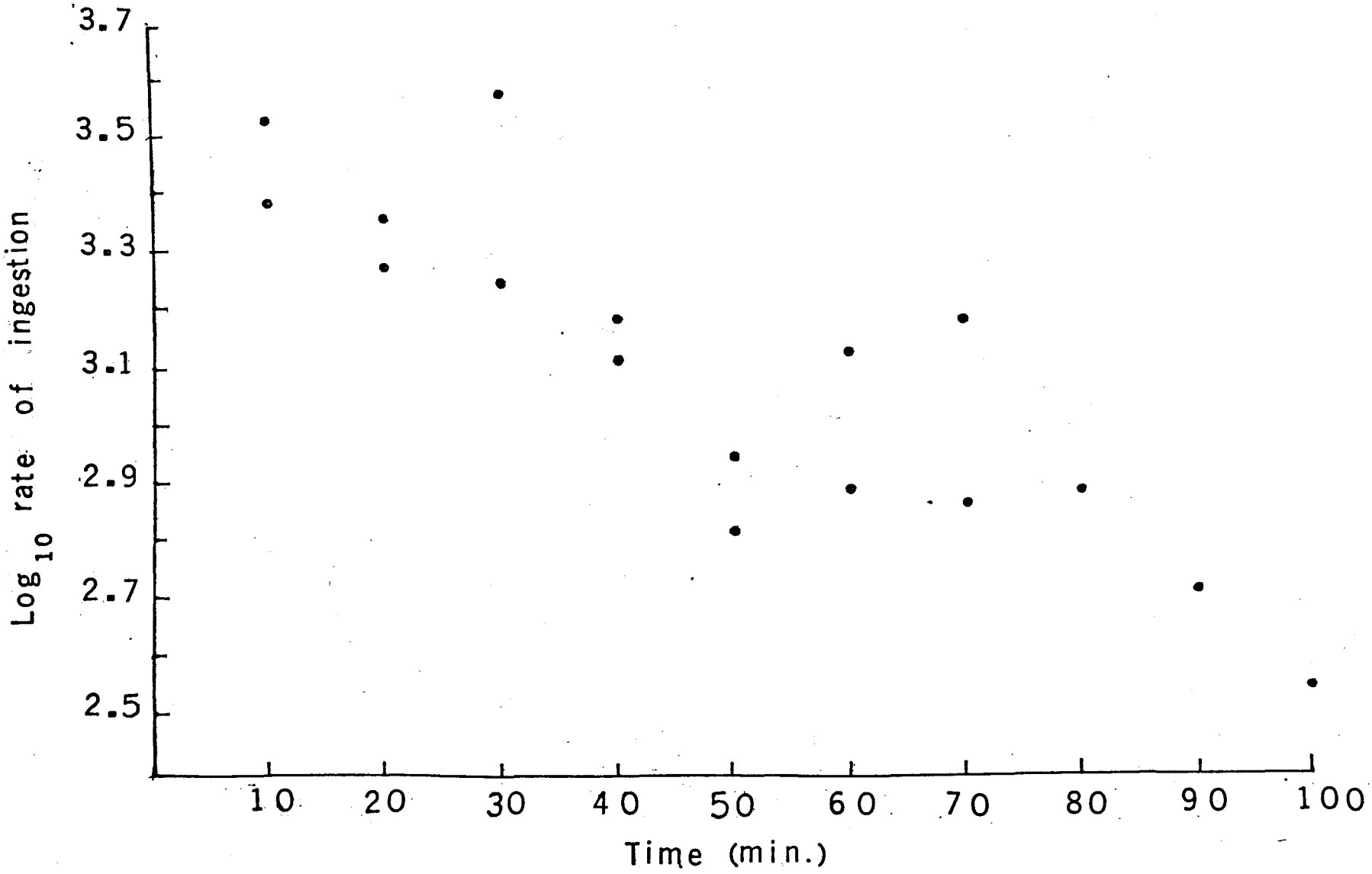

m
tr
A
2 mm.
B

Figure 3

Plot of $\log_{10}$ rate of ingestion (cells min^{-1} $zooid^{-1}$) of L. carteri vs. time in minutes.


3.7
3.5
3.3
3.1
2.9
2.7
2.5
Log10 rate of ingestion
10
20
30
40
50
60
70
80
90
100
Time (min.)

Literature Cited

Atkins, Daphne. 1932. The ciliary feeding mechanism of the entoproct polyzoa and a comparison with that of the ectoproct polyzoa. Quart. J. Micros. Sci. 299: 393-423.

Bidder, G. P. 1923. The relation of the form of a sponge to its currents. Quart. J. Micros. Sci. 67: 293-323.

Bullivant, J. S. 1967. Aspects of feeding of the bryozoan Zoobotryon verticillatum (delle Chiaje). Ph.D. Thesis, University of Southern California.

Gauld, D. T. 1951. The grazing rate of planktonic copepods. J. Mar. Biol. Assoc. U. K. 29: 695-706.

Gibb, J. A. 1962. L. Tinbergen's hypothesis of the role of specific search images. Ibis. 104: 106-111.

Holling, C. S. 1965. The functional response of predators to prey density and its role in mimicry and population regulation. Mem. Ent. Soc. Can. 45.

Huffaker, C. B. 1958. Experimental studies on predation: dispersion factors and predator-prey oscillations. Hilgardia. 27: 343-383.

Hyman, Libbie H. 1959. The Invertebrates: Vol. V. Mcgraw-Hill Book Co., New York.

Ivlev, V. S. 1961. Experimental Ecology of the Feeding of Fishes. Yale Univ. Press, New Haven. 302 p.

Jørgensen, C. B. 1966a. Biology of Suspension Feeding. Pergamon Press, New York. 357 p.

———————, 1966b. Feeding. chap. II In Marine Biology III, Proceedings of the Third International Conference. W. T. Edmondson, (ed.), New York Academy of Sciences Interdisciplinary Communications Program, New York: 69-133.

Rogick, Mary D. 1937. Culturing freshwater Bryozoa. In Culture Methods for Invertebrate Animals. P. Galtsoff et al (eds.), Dover Publ.

Ryther, J. H. 1954. Inhibitory effects of phytoplankton upon the feeding of Daphnia magna with reference to growth, reproduction, and survival. Ecology. 35: 522-533.

Vita

Leonard M. Bahr, Jr. was born on June 17, 1940, in Baltimore, Maryland. He received his primary education in public schools in Baltimore and Elkridge, Maryland, and in 1954 he received an academic scholarship to the McDonogh School, in Baltimore County. He was named a runner-up in the National Merit Scholarship Competition in 1958. At that time he entered the University of Maryland, and in 1963 he graduated with a B.S. degree in Zoology. He accepted a position as Faculty Research Assistant at the Chesapeake Biological Laboratory of the University of Maryland Natural Resources Institute in Solomons, Maryland. Until 1966, he was engaged in research on shellfish (oyster) related problems that led to two publications. In 1966 he entered the University of Richmond to begin work on an M.S. degree in biology. While in Richmond he married the former Susan W. Clark, in March, 1967. He received the degree of Master of Science in August, 1968. In September, 1968, he plans to enter the University of Georgia to continue his graduate studies toward a Doctor of Philosophy degree in marine ecology. He has been awarded a teaching assistantship.