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Studies on the Functional Role of Tintinnids in the Southern California Bight. II. Grazing Rates of Field Populations

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Abstract

The feeding behavior of relatively undisturbed natural populations of tintinnids was studied in a series of experiments which utilized water samples collected in Southern California coastal waters. Dilute suspensions of corn starch were presented to the tintinnids and the rates of ingestion of this visual tracer determined after short (5 to 20 min) incubations followed by fixation in Lugol's iodine fixative. Tintinnids were observed to ingest particles of diameters up to approximately 43% of their lorica oral diameter and at rates proportional to their oral diameters. Grazing rates observed in these experiments ranged up to approximately 10 µl tintinnid^{-1} h^{-1}, and generally agreed well with the rates observed in experiments utilizing laboratory cultures of tintinnids. No significant diel periodicity in feeding rates was observed in the one study extending over a 24 h period.

Introduction

Despite intensive efforts in the past several decades to study the flow of energy and materials through and within plankton communities, it is only quite recently that any substantial attention has been paid to that diverse fraction of the assemblage collectively defined as the micro-zooplankton. To date, most research on the micro-zooplankton has been devoted to defining distribution and abundance patterns (e.g. Beers and Stewart, 1967, 1969, 1970; Beers *et al.*, 1971, 1975), with relatively less effort being devoted to such topics as metabolic or trophic activities.

With the development of techniques for the laboratory culturing of tintinnids (Gold, 1968), it has become possible to carry out physiological investigations with these organisms under controlled conditions. A series of such experiments, designed to assess the feeding and growth rates of several neritic tintinnids, largely as functions of food concentration, has recently been reported (Heinbokel, 1978). While these experiments yielded results which seemed reasonable in both form and magnitude, the applicability of these results to the natural field situation remained untested. In addition to potentially biasing differences from field conditions in such controlled parameters as light, temperature, turbulence, and food type and concentration, which influence all such laboratory determinations, these experiments were performed exclusively with tintinnids which had been grown for a minimum of 25, and more often 50 to 100, generations in the laboratory. The potential for adaptation to or selection for success in such artificial culture conditions is obvious, and requires consideration before the results of these culture experiments can be applied to an analysis of the natural system.

Accordingly, an effort was made to evaluate the grazing rates obtained in the culture experiments by making similar determinations on natural assemblages of tintinnids. This was accomplished by the short-term monitoring of the ingestion of visually distinct particulate material which was added to otherwise untreated natural water samples (Spittler, 1973). These experiments also allowed three observations which were not feasible in the laboratory experiments. Specifically, the size range of particles (corn starch with a range of particle diameters of approximately 2.5 to 25 µm) used in these experiments permitted the determination of the size of particles which could be ingested by

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various species of tintinnids; the presence of several species of tintinnids which had not been cultured for the earlier experiments allowed at least a preliminary evaluation of grazing rate as a function of grazer size; and finally, this procedure was used to determine if any marked diel periodicity existed in the grazing rates of the natural tintinnid populations.

Materials and Methods

These experiments were closely patterned after those of Spittler (1973). For each experiment, a suspension of corn starch was prepared in deionized water and the concentration and size spectrum of particles determined with a model TA II Coulter Counter using a 100 µm orifice. The stock suspension was then refrigerated and used for all grazing rate determinations of that experiment; re-measurement of the suspensions at the end of each experiment never revealed any significant change from the initial characteristics. For each grazing rate determination, 2 to 4 screw-cap glass jars were used. A measured aliquot of the thoroughly mixed corn starch suspension was placed into each jar followed by the addition of 300 to 800 ml of unfiltered surface sea-water (with its natural component of tintinnids, phytoplankton, and other seston) which had been collected from near the pier of the Scripps Institution of Oceanography, La Jolla, California, USA. Ambient water temperatures ranged between 17° and 20°C in these experiments. This sea-water was similarly examined with the Coulter Counter (100 µm orifice) to determine the natural particle concentration and size spectrum. The bottles were then rapidly capped and gently rotated about their long axes in a constant temperature room at 18°C ± 0.5 C°. At timed intervals (from 5 to 30 min after inoculation of the sea-water with the starch), bottles were removed from the grazing wheel and their contents fixed with Lugol's iodine fixative. A portion of each fixed sample was then gently poured through 20 µm mesh netting and the retained material washed into a 10 ml settling chamber and examined with a Unitron inverted microscope. Each tintinnid was identified to species, the oral diameter of the lorica measured, and the number and maximum diameter of ingested starch grains (which appeared black in the iodine fixative) determined.

From these data the ingestion rate (I, as starch grains tintinnid^{-1} h^{-1}) could be determined. Then, assuming that the largest particle which had been ingested represented the maximum which could be ingested, and knowing the concentration of starch grains (P_S, as grains ml^{-1}) less than or equal to this critical diameter, the grazing rate (F, as µl tintinnid^{-1} h^{-1}) of that particular species could be calculated:

$$F = \frac{I}{P_S} \cdot$$

The experiments performed are summarized in Table 1.

Table 1. Summary of experiments measuring ingestion by natural assemblages of tintinnids

Experiment no.	Date (1976) and time	Tintinnids present	Particulate material available (No. ml^{-1})		
			Natural	Corn starch	Total
10	23 July	*Tintinnopsis* cf. *acuminata*	14,740	6,495	21,235
	11.00 hrs	*Amphorella quadrilineata*	14,804	6,563	21,367
		various other species			
11	26 July	*T.* cf. *acuminata*	21,994	5,782	27,776
	11.00 hrs	*Eutintinnus pectinis*	21,994	5,782	27,776
		various other species			
	23.00 hrs	*T.* cf. *acuminata*	24,923	5,797	30,720
		E. pectinis	24,923	5,797	30,720
		various other species			
12	2 Aug.	*T.* cf. *acuminata*	20,620	3,353	24,173
	11.00 hrs	*Amphorellopsis acuta*	21,201	3,437	24,638
13	30 Nov.	*Salpingella curta*	5,520	1,742	7,262
	11.00 hrs	*T.* cf. *acuminata*	5,612	1,852	7,464
		E. pectinis	5,612	1,852	7,464
		Helicostomella subulata	5,612	1,852	7,464
		A. quadrilineata	5,650	1,996	7,647
		A. acuta	5,650	1,996	7,647
	15.00 hrs 19.00 hrs 23.00 hrs 03.00 hrs 07.00 hrs	All parameters approximately as for the first samples at 11.00 hrs			

Results and Discussion

Size of Ingested Particles

In order to evaluate the feeding impact of tintinnids under natural conditions, it is essential to know what fraction of the particulate material present is actually available to the grazers. Two previous studies (Spittler, 1973; Blackbourn, 1974) addressed this topic, and similar data were obtained from the present study (Fig. 1). Spittler (1973), working with natural assemblages of tintinnids in the Baltic Sea, reported that the largest ingested particles consistently had diameters of 41 to 45% of the oral diameter of the lorica. Blackbourn (1974) similarly measured the largest ingested particles for a variety of tintinnids exposed to a wide range of food types and sizes. His data (presented as particle volume graphed as a function of tintinnid body volume) have been converted (assuming all the particles to be spherical) to the units used by Spittler (1973) and this study, and are also presented in Fig. 1. Those data, and the data collected in this study, agree with those of Spittler (1973) in showing that a wide variety of tintinnids are capable of ingesting particles whose diameters approach 40 to 45% of the oral diameter of the tintinnid.

Considerably less is known of the grazing abilities of tintinnids on smaller particles of the food spectrum. Spittler (1973) presented evidence that tintinnids would not ingest particles less than 2 µm in diameter except under conditions of temperature stress. Blackbourn (1974), however, reported a number of tintinnids were able to ingest 1 µm latex spheres. In the discussions which follow, the assumption is made that all particles up to a critical maximum size (43% of the lorica oral diameter) are ingested with equal efficiency. While this assumption is certainly an oversimplification, the data are insufficient to define a more realistic relationship. Similarly ignored in the following analyses are other factors which could be expected to influence "ingestibility", such as particle shape and motility.

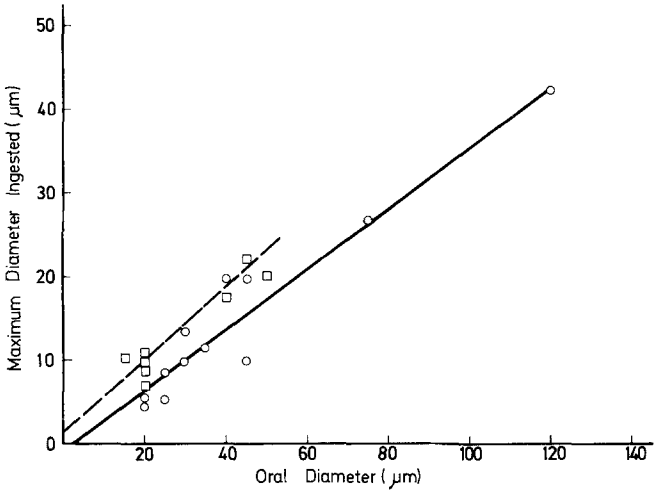

Fig. 1. Maximum particle diameters observed to have been ingested by tintinnids as a function of the lorica oral diameter of the tintinnid. Circles: data from Blackbourn (1974), $Y = (0.37 \cdot X) - 0.75$; squares: data from this study, $Y = (0.43 \cdot X) + 1.22$

Ingestion of Particles by Field Populations

Two problems were encountered in adapting Spittler's technique for use in this study. The first of these was in determining the proper combinations of corn starch concentrations and incubation times which would result in the most precise and accurate estimates of grazing. Too short an incubation and/or too little corn starch results in low numbers of ingested particles with correspondingly low precision in feeding rate estimates. Too long an incubation leads to the possibility that digestion and/or egestion will interfere with the estimation of feeding rates, while too high a concentration of corn starch results in tintinnids so fully packed with starch grains that accurate counting is impossible. The results to be reported here are from those uptake experiments which lasted no longer than 20 min, a period of time in which the rate of corn starch accumulation remained constant.

A second problem involved the interpretation of the ingestion rates. If the rate at which the tintinnids were feeding depended on the recent history of food conditions, then the observed rate should depend on the concentration of natural particles alone; whereas, if the feeding rate was determined by the conditions at that precise moment, then the observed rate should be related to the sum of natural particles and the added corn starch. To determine which model was the better one, 6 additional bottles were inoculated at the start of Experiment 11 with both the corn starch and an aliquot of *Isochrysis galbana* culture sufficient to achieve a concentration of approximately 20,000 *I. galbana* ml^{-1}. With both *Eutintinnus pectinis* and *Tintinnopsis* cf. *acuminata* present in all the bottles, this resulted in 12 comparisons of grazing rates between *I. galbana*-enriched and non-enriched treatments. In 10 of these

comparisons, the tintinnids in the bottles containing the *I. galbana* displayed lower grazing rates than the comparable but non-enriched treatments, suggesting that the immediate conditions were more important than recent history in determining grazing rates. A second explanation for these data is possible. An instance of apparently selective feeding observed for *E. pectinis* during the culture experiments (Heinbokel, 1978) suggests that the depressed grazing rates on corn starch observed in the enriched bottles might represent not an overall lowering of grazing rate but rather a selection against the corn starch with increasing levels of "real" food. There is no way at present to discriminate between these two explanations; for consistency throughout this discussion the observed grazing rates will be related to the total particle concentration (natural material plus corn starch) in the grazing bottles.

Three aspects of the feeding dynamics of tintinnids were examined in these experiments. The first of these dealt with the functional responses of two species of tintinnids, *Eutintinnus pectinis* and *Tintinnopsis* cf. *acuminata* (Fig. 2). The latter species was present in all the field experiments and was, therefore, exposed to a wide range of particle concentrations during the course of the 4 experiments; the former was present in 2 experiments. In this analysis, only those particles were considered which had equivalent diameters between 2.5 µm (the lowest channel of the Coulter counter) and 10 µm, the largest size which could be ingested by these particular species. It is difficult to compare these results directly with those of the laboratory cultures of these species, since the available food in the two cases are quite different and are measured on different scales. Despite these difficulties in interpretation, it is gratifying to note that for *T.* cf. *acuminata* the highest observed grazing rates of the field populations closely match the highest rate noted in the laboratory (2.5 µl tintinnid^{-1} h^{-1}; Heinbokel, 1978) and that the grazing rates follow the general pattern of a continuous decrease with increasing food concentrations. Fewer data are available for *E. pectinis*, but a similar pattern of decreasing grazing with increasing particulate material can be seen. The maximum grazing rate of approximately 3 µl tintinnid^{-1} h^{-1} observed in culture experiments (Heinbokel, 1978) is, however, somewhat lower than the maximum rate displayed by the field populations. Here again, an alternate mechanism based on selective feeding as

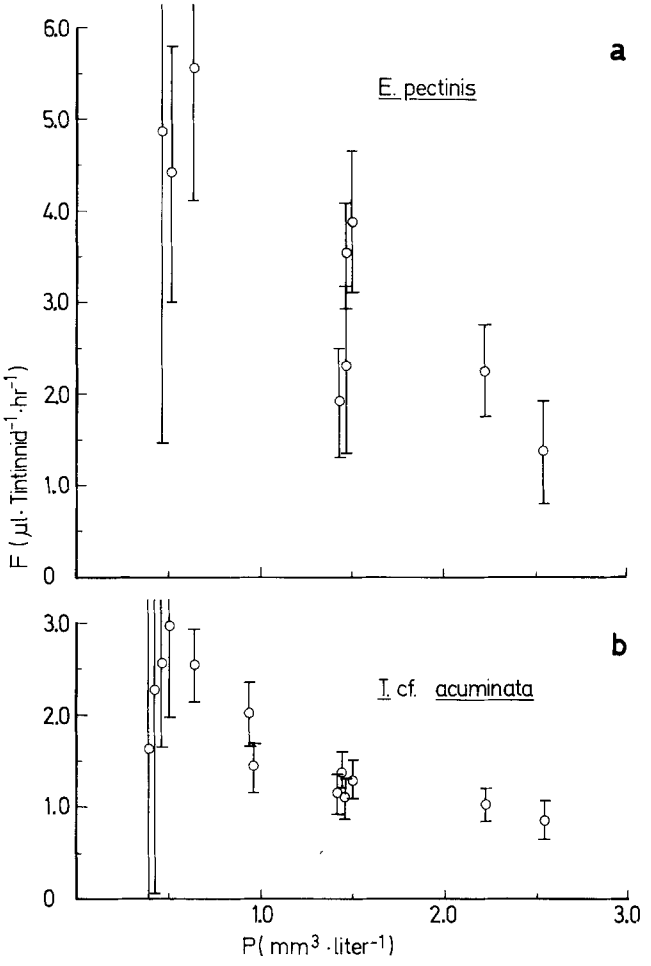

Fig. 2. *Eutintinnus pectinis* (a) and *Tintinnopsis* cf. *acuminata* (b). Volume swept clear of corn starch (*F*) as function of food concentration (*P*). Error bars represent 95% confidence limits of the individual estimates

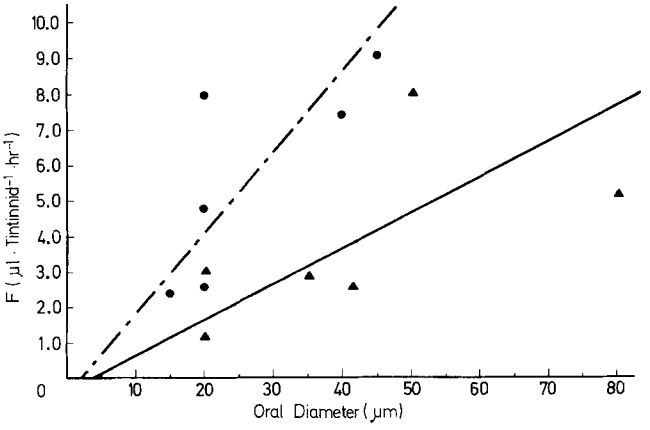

Fig. 3. Volume swept clear of corn starch (*F*) as function of the oral diameter of several species of tintinnids. Triangles: Experiment 11, $Y = (0.10 \cdot X) - 0.33$; circles: Experiment 13, $Y = (0.23 \cdot X) - 0.42$

discussed above could account for the shape of the observed functional response in the field populations of these two species.

A second aspect of tintinnid feeding behavior which is especially important when applying experimental data to the field situation is the relationship of tintinnid size to grazing rate. Since only a few species of tintinnids have been cultured for such experimental determinations (Heinbokel, 1978), it is necessary to have some rational means for extending and applying these data to the many other species encountered in natural waters. The oral diameter of the lorica has been shown to be one of the most conservative dimensional characteristics of the individual tintinnid species (Kofoid and Campbell, 1939; Blackbourn, 1974; Gold and Morales, 1975). Since oral diameter has already been shown to be correlated with the maximum size of ingested particle, and can be related to the grazing rates determined in the culture experiments (Heinbokel, 1978), the observed grazing rates from these corn starch ingestion experiments were examined for any relationship with the oral diameters of the tintinnids present. Two experiments (11 and 13) contained a sufficient number of tintinnid species to allow this type of analysis. Since the two experiments differed considerably in the amount of natural particulate material present, they were analyzed separately (Fig. 3). In each experiment, the grazing rate was calculated for each of the 6 species present and plotted as a function of lorica oral diameter for that species. Bartlett (1949) regression lines were then calculated for each experiment. Neither set of data yields a particularly precise estimate of the apparent relationship, but in both cases the slopes are significantly different from 0 ($P < 0.05$). Neither Y-intercept differs significantly from 0, and confidence limits for the slopes overlap broadly, suggesting an overall similarity in response between the two experiments.

An additional purpose of Experiment 13 was to determine if there was any indication of diel periodicity in the feeding behavior of tintinnids. Although Blackbourn (1974) stated that light level did not influence grazing rates in his experiments, Campbell (1926) and Biernacka (1952) both indicated that natural tintinnid populations appeared to be synchronized in the timing of division. Such synchrony, if also present in feeding behavior, would significantly influence any evaluation of the role of the tintinnids in the plankton. Surface samples were taken every 4 h from the end of the Scripps pier for a 24 h period to examine this question. Six species of tintinnids were present in sufficient numbers to estimate their grazing rates, although only 3 species were present at all 6 sampling times. The results (Fig. 4) show no significant diel periodicity in the feeding behavior of the total assemblage which could be adequately separated from the large variabilities inherent in the individual grazing estimates.

Comparison with Laboratory Cultures

One of the major goals of these experiments was to compare the grazing rates of minimally disturbed field populations with the results obtained in simplified laboratory systems and with tintinnid populations which had experienced strong selective pressure for rapid growth under these artificial conditions. Two species (*Eutintinnus pectinis* and *T.* cf. *acuminata*) which had been extensively cultured and which were present in many of the field experiments have been discussed above. Quite reasonable agreement between the determinations was found. *Helicostomella subulata,* a third extensively cultured species (Heinbokel, 1978), was present in only one field experiment (Experiment 13). It displayed grazing rates higher than those observed in the culture studies (approximately 8 µl tintinnid^{-1} h^{-1} in the field experiments compared to a maximum of approximately 4.7 µl tintinnid^{-1} h^{-1} in the culture experiments; Heinbokel, 1978). Overall though, assuming that the ingestion of corn starch by field populations is actually measuring the same process as is measured by the laboratory experiments, it appears that, while maximum grazing rates may be somewhat lower in the laboratory system than under more natural conditions, both the magnitude and shape of the functional response have been adequately defined in the laboratory.

In order to evaluate this last assumption, a single experiment was performed in which a laboratory culture of *Eutintinnus pectinis* was used to obtain grazing rate estimates by both methods (Fig. 5). Three "grazing" and two control bottles were set up for each of two initial concentrations of *Isochrysis galbana* and grazing rates determined during 4 sampling intervals as described previously (Heinbokel, 1978). At the end of each of the last two intervals, one grazing bottle from each treatment set was inoculated with a dilute suspension of corn starch. Samples were drawn from these bottles 5, 10, and 20 min after inoculation, fixed

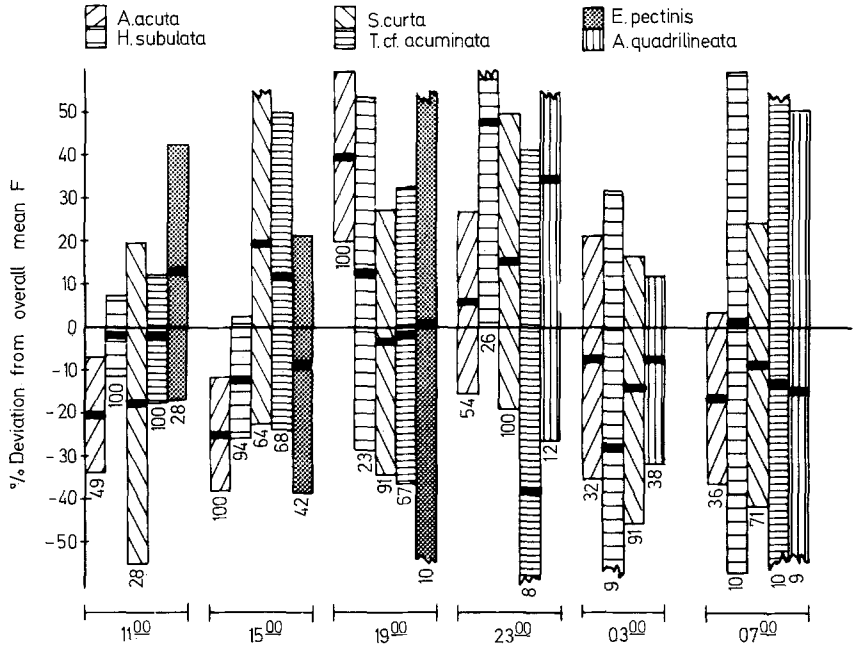

Fig. 4. *Amphorellopsis acuta, Helicostomella subulata, Salpingella curta, Tintinnopsis* cf. *acuminata, Eutintinnus pectinis,* and *Amphorella quadrilineata*. Volume swept clear of corn starch by tintinnids of a natural assemblage (Experiment 13). Estimates of grazing rates were made 6 times during a 24 h period. Grazing rate (solid bar) is presented for each species present at each sampling time as percent deviation from the overall mean rate observed for that species through the entire experiment. Bars identify the species and represent the 95% confidence limits of the individual estimates. Numbers beneath bars denote number of individual tintinnids examined to obtain each estimate

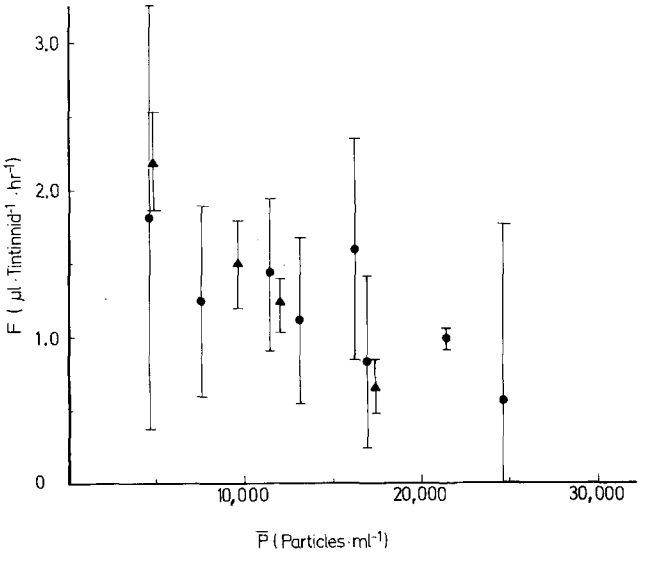

Fig. 5. *Eutintinnus pectinis*. Volume swept clear (F) as function of particle concentration ($\bar{P}$), as estimated by two methods in a single experiment using a laboratory culture of *E. pectinis*. Circles denote estimates obtained by standard cell count method of Heinbokel (1978); triangles denote estimates obtained by short-term observation of the ingestion of corn starch. Error bars represent 95% confidence limits of each estimate

with Lugol's fixative, and grazing rates determined as described above. The grazing rates as determined by the ingestion of the corn starch appear to decrease more rapidly with increasing food concentration than do the comparable laboratory estimates, although there is considerable overlap in the confidence intervals at any given food concentration. A rigorous analysis of this apparent difference is not possible because of the low precision of the laboratory estimates and uncertainty as to the form of the equation with which to model the grazing function (e.g. Frost, 1975). In spite of these problems, however, it appears that any differences in the two types of estimates could be explained by the selective feeding noted previously for this species of tintinnid (Heinbokel, 1978). This, combined with the overall similarity in grazing rates obtained with the two methods, suggests that the two measurements are indeed providing equivalent estimates of grazing rates.

Conclusions

The present study was undertaken primarily to evaluate the relevance of grazing

rates obtained using laboratory cultures of tintinnids. The results of these experiments utilizing natural field assemblages agree well with the laboratory determinations, lending credence to the generally more precise data which can be obtained from the simplified conditions of the culture experiments.

In addition, and perhaps ultimately of more general importance, such experiments with natural populations provide a means to ask questions which would be extremely difficult to address with laboratory cultures. Uncertainties as to exactly what portion of the natural particle spectrum is truly available to a particular grazer and as to the prevalence of selective feeding may often limit such investigations to determining relative rather than absolute rates, but this only slightly weakens the potential of such studies. Potential applications of these experimental methods include: (1) the study of grazing processes of many ciliate species which are not available in laboratory culture; (2) the determination of the size range of food particles which can be utilized by a particular species; (3) the determination of grazing rates along various temporal or spatial scales, e.g. the single study of diel grazing periodicity presented in this paper; (4) the study of the relationship of grazing rate and food concentration in natural populations of grazers through the use of appropriate dilutions or enrichments; (5) the study of the acute effects of pollutant or temperature stress on natural assemblages.

In short, the experimental use of both laboratory cultures and field populations provides complementary data which should allow wide-ranging investigations into the activities and roles of this largely neglected component of the zooplankton.

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