

ZOOPLANKTON GRAZING IN
KANEHOHE BAY, HAWAII

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ABSTRACT

Grazing rates of several abundant zooplankters in Kaneohe Bay, Hawaii were measured at different concentrations of natural phytoplankton. The concentration by volume of suspended particles, as determined with an electronic particle counter, was used as the estimate of food concentration. The relationship between grazing rate per animal and concentration of particulate food conformed closely to a hyperbolic model widely used to describe an organism's rate of uptake of food or other needed substrate as a function of the concentration of the substrate. Maximum observed grazing rates in the eutrophic south sector of the bay are near the maximum rates predicted by the model. The concentrations of particles in other areas of Kaneohe Bay do not appear to be high enough to permit grazing rates to approach their maximum levels. There appears to be no preference by the grazers for particles of a size other than the size most abundant in the environment.

INTRODUCTION

Although grazing by plankton in the sea has long been an object of interest and experiment, Cushing concluded as late as 1958 that experimental work had not yet been sufficient for development of a theoretical model of the process. Work in other environments and with non-planktonic animals, however, had provided a foundation for a description of the general process of an organism's acquisition of food or other needed substrate from its environment.

Gause (1934), for example, used an equation of the form

$$r = R(1 - e^{-kp}) \quad (1)$$

to describe the relationship between predator growth rate (r) and prey abundance (p), where R is the maximum specific growth rate and k is a constant. An equation of the same form was later used by Ivlev (1961) to describe the feeding rate (r) of fishes at various food concentrations (p). A modified form of (1) has recently been applied to zooplankton grazing (Parsons, LeBrasseur, and Fulton 1967), and Conover (1968) has discussed several such applications.

Hinshelwood (1946) discussed a model which applied principles of chemical reaction rate kinetics to the substrate-limited growth of bacteria. The relationship between growth (or uptake) rate and substrate concentration

is described by the Michaelis-Menten hyperbola

$$r = R(S/(K+S)) \quad (2)$$

where r and R are as in (1), S is the substrate concentration (comparable to p above), and K is a constant equivalent to the value of S for which $r = R/2$. Caperon (1967) applied this model to phytoplankton and substrates limiting their growth, and suggested that the model may apply to higher trophic levels.

Holling (1959) derived a model equivalent to (2) for the "functional" (change in feeding rate) response of predators in general to changes in prey density, and applied his model to data obtained from a study of a host-parasite interaction, and later (Holling 1965) to a study of an insect predator and its prey.

An experimental comparison of models (1) and (2) is not attempted in this study. Holling (1965), Rashevsky (1959), and Royama (1971) have compared them and discussed their applications. Both models describe the specific growth or uptake rate as increasing with increasing substrate (food) concentrations, but at a decreasing rate, eventually approaching an asymptote (R).

Caperon, Cattell, and Krasnick (1971) have used the hyperbolic model to describe the relationship between planktonic trophic levels in Kaneohe Bay, Hawaii. For a steady-state ecosystem with all "substrate" concentrations (nutrients and the standing stocks of the organisms in the

various trophic levels) in the low range, that is, in an oligotrophic system, any change in input, such as the supply of plant nutrients in sewage effluent, is balanced primarily by changes in uptake or feeding rates (functional response), a relatively rapid process. For a steady-state eutrophic system, substrate concentrations are high; the response to changes in substrate concentrations can only be changes in the standing stock of the organisms using a substrate (numerical response), a relatively slow process. The slowness of the numerical response causes short-term departures from the expected steady state in the form of fluctuations in the substrate concentrations. The authors have observed such fluctuations, which would not appear in an oligotrophic system, in both nutrient concentrations and phytoplankton standing stock in Kaneohe Bay, and they showed that the observed nutrient concentrations are high enough to make a functional response impossible for many phytoplankton species. By similar reasoning, they infer that the fluctuations in phytoplankton standing stock result from the grazers' inability to make the more rapid functional response to changing phytoplankton concentrations.

The purpose of the experiments described here was to study empirically the relationship between grazing rate and food concentration for some of the grazers in Kaneohe Bay, in order to determine whether the above inference is correct.

Studies of this relationship are now abundant in the literature, but most, like those of Anraku (1964) and Mullin (1963) have used laboratory cultures of algae as food. A recent example of an application of a mathematical description to data from experiments done with natural phytoplankton is the work of Parsons, et al. (1969). A similar approach, with some modification, has been adopted for this study.

METHODS

Experimental animals were obtained in net tows at the surface in the south sector of Kaneohe Bay. The tow sample was poured through a set of plankton-mesh filters of different pore sizes, and the retained animals were washed with filtered bay water. This procedure removed phytoplankton and detritus that had been caught in the tow sample, and produced a suspension of fairly uniform-sized animals (less than one mm long), mainly the copepods Paracalanus parvus and Oithona sp. (adults and late stages) with occasional small barnacle nauplii. These animals appear to be the dominant grazers in this region of the bay. Both Clutter (1969) and Peterson (1969) found that the "micro-copepods" (those passing through 333-micron mesh) were far more numerous (more than 100/l) than all larger grazers taken together, and Clutter also found that the microzooplankton usually exceeded the large forms (including carnivores) in wet volume. Copepod nauplii were sometimes abundant in tow samples, but they were too small to be counted and handled in these experiments.

Each experiment involved four pairs (experimental and control) of 2800ml wide-mouth erlenmeyer flasks, each flask containing one liter of water. All water used was passed through 100-micron mesh to remove grazers present in the water sample. One pair of flasks in each experiment

contained unmixed natural water. Other concentrations of suspended particles were produced by mixing surface bay water (taken at the time and site of the plankton tow) in various proportions with bay water filtered through Whatman GFC glass fiber filters.

Animals from the prepared suspensions were drawn up in a pipette, counted in the pipette under a dissecting microscope, and placed into the experimental flask of each pair. The number of animals per flask (50-100) was chosen in an attempt to approximate the biomass per experimental volume suggested by Sheldon and Parsons (1967), that is, 2-20mg wet weight/l, without exceeding the environmental abundance mentioned above. Since the average weight of an animal from the suspension was about 23 μ g (estimated from Clutter's 1969 data), the biomass of grazers in an experimental flask was about 1-2mg. The flasks were incubated overnight (15-25 hrs) in near-darkness on a shaker table. Water temperatures in the flasks were 26-27 C and did not change appreciably during incubation.

Initial and final counts of the particle concentration in all experimental and control flasks were made with a Cell-O-Scope electronic particle counter. At least three replicates were made of each count; the volume of a counted sample was either 0.5 or 2.0ml. This instrument, like the model B Coulter counter (Sheldon and Parsons 1967), counts all suspended particles in a given size range and

analyzes the distribution of the total count over this size range. These numerical distributions (fig. 1A) were converted to distributions of suspended particle volume (fig. 1B). The size range of particles counted, which is determined by the size of the orifice through which the sample is drawn, and by the sensitivity settings of the instrument, was chosen to include as much as possible of the suspended particle volume, as shown in fig. 1B. All particles are represented as having a certain diameter, but this is actually the diameter of a sphere having a volume equivalent to that of the counted particle.

RESULTS

The size distributions of suspended particles and microscopic examination of the water samples indicated that most of the particles dealt with in these experiments were chains of various lengths of the diatoms Skeletonema costatum and several species of Chaetoceros. It was estimated that a sphere of 31 microns diameter (the size most abundant by volume in fig. 1B) has a volume (1.6×10^4 cubic microns) equivalent to that of a chain 30-50 cells long of a small species of Chaetoceros.

The differences in suspended particle volume concentration between experimental and control flasks at the time of the final counts was tested for significance by the Wilcoxon Signed-Rank Test (Tate and Clelland 1957). The paired observations required were the particle volumes (V_c = control, V_e = experimental) in each size class for a pair of flasks. The test was made over all the size classes distinguished by the instrument (128), although the classes have been grouped for presentation in fig. 1. For all but two of the 31 pairs of flasks involved in the series of experiments, the difference ($\sum V_c - \sum V_e$) was positive and significant ($p < .05$, one-tailed test). In both exceptional cases, the difference was positive but of less definite significance ($p < .10$).

The measure of grazing was taken as the sum over the

size classes of the positive ($V_c - V_e > 0$) size-class differences. The reasons for this procedure and its implications will be discussed later. The "food concentration" in an experiment was taken as the mean of the initial and final experimental concentrations. With one exception, the final concentration was never less than 50% of the initial concentration (mean: 75.4%). The results of several experiments are presented in fig. 2. The curves are least-squares fits of equation (2) to the data.

As mentioned, the maximum particle concentration in each experiment was that of the natural water, after the screening to remove zooplankton. Table 1 shows that the grazing rates at the maximum food concentrations were, with one exception, greater than 90% of the predicted maximum rates.

Fig. 3 shows the correspondence between the particle size most abundant by volume in the water and the size grazed most heavily. Although the correlation is highly significant, and the 95% confidence interval for the size grazed most heavily (35.1 ± 3.2 microns) includes the mean size at peak abundance in the water (38.1 microns), the mean difference in the two peak sizes is significant.

DISCUSSION

Several processes other than grazing can cause changes in concentrations of suspended particles during experiments such as these. Mullin (1963), Anraku (1964), and Sheldon and Parsons (1967) have discussed various problems and influential factors to be considered in grazing experiments. Some of these should be considered with respect to the work presented here. Except as noted, the use of control flasks, rather than before and after measurements, was relied upon to help eliminate errors from these factors.

1) Settling of particles. Tests showed that the shaker table kept particles well-suspended during incubation compared to particles in flasks not placed on the apparatus.

2) Plant growth and particle formation. For some experiments, the final particle volumes in the control flasks exceeded that in the original water. It has been assumed that the experimental flasks were affected similarly in these cases. The assumption is reasonable, since growth effects appeared to be consistent within experiments.

3) Nauplii. For some experiments, the final control particle volume was less than that in the original water. This may be attributed in part to the grazing activity of tiny copepod nauplii, which were sometimes observed in water that had passed through 100-micron mesh. Screening

through finer mesh to remove such small animals would have caused unacceptably large changes in the amount and size distribution of food particles.

4) Breaking of large particles. If a large particle (such as a diatom chain) is broken during feeding, but not all fragments are eaten, the volume of the large particle (which is removed from its size class) is an overestimate of the amount grazed. The ungrazed fragments are added to the distribution in the smaller size classes, and create negative volume differences ($V_c - V_e < 0$) in those size classes. As mentioned, however, negative differences have been ignored in the measure of grazing, so the measure is likely to be an overestimate. The use of control flasks does not, of course, compensate the effect of the breaking of particles by grazers in the experimental flasks.

5) Fecal pellet production. Large negative volume differences can result from production of fecal pellets in the experimental flasks, as well as from fragments of broken particles. For some pairs, in fact, the negative differences were a sizeable fraction of the positive differences. Although this fraction was quite variable, the negative differences were generally greater at high food concentrations and grazing rates, as would be expected if these differences are partly caused by fecal pellets. The bias which would result from such a trend was considered sufficient reason to ignore the negative differences. The

extent of the overestimate of grazing rates due to factor 4) above, however, remains unknown, and depends on just how much of a broken diatom chain is eventually eaten.

For comparison with other work, note that 10^6 cubic microns of water weighs about $1\mu\text{g}$. Estimates of dry weight or carbon content of living matter from wet weight or cell volume are discussed by Jorgensen (1966), by Mullin, Sloan, and Eppley (1966), and by Sheldon and Parsons (1967). The grazing rates observed in this study were very similar to those reported by Parsons, et al. (1969) for experiments with Pseudocalanus and Oithona, and to the observations of Hargrave and Geen (1970) for Pseudocalanus and Acartia. Both studies used naturally occurring phytoplankton as food.

The correlation demonstrated in fig. 3 should be interpreted with caution. If there were no discernible peak in the particle volume distributions for the water, then a sharp and consistently located peak in the grazed volume distributions could reasonably be taken as indicating a preference by the grazers for particles of that size. But the peak for the water is distinct (as in fig. 1B), though somewhat variable (range: 28-56 microns, s.d.: 6.77 microns), as is the peak in grazed volume. Although the mean difference in particle size at the two peaks (3.0 microns) is significant as mentioned, fig. 3 shows that the good correspondence between the peak sizes exists over the entire range of peak particle sizes. It is therefore

reasonable to conclude that there is not a preference for particles of a size greatly different from the one most abundant in the water. It was not determined whether the most abundant sizes were grazed in proportions equal to or greater than their abundance, but the lack of preference for other particle sizes suggests that these grazers are well-adapted to the food available to them. This conclusion conflicts with Peterson's (1969) contention that the chain-forming diatoms in Kaneohe Bay are not suitable food for the microzooplankton.

Table 1 and fig. 2 indicate that the environmental concentration of phytoplankton in the south sector of Kaneohe Bay during this study was usually high enough for Paracalanus and Oithona to graze at a rate near the theoretical maximum. That is, a functional response by the grazers to an increase in food concentration was not possible, as Caperon, et al. (1971) hypothesized. The means of the observed and predicted maximum grazing rates do not differ significantly (paired-sample t-test, $p > .50$).

The maximum observed grazing rates do, however, vary over a rather wide range, and they are positively correlated with the maximum food concentrations in each experiment (fig. 4). This suggests that the "maximum" grazing rate determined in short-term experiments might be influenced by preconditioning at different food concentrations. Reeve (1964) noted that the maximum feeding rate of Artemia

in his experiments was not constant, and he discussed how adjustments in this maximum rate with changing conditions might be advantageous to the animal. The relationship shown in fig. 4 is insufficient to support a hypothesis of preconditioning in these experiments, because the concentrations of food in the water during the several days preceeding each experiment were not known.

Although the values of K derived from individual experiments are variable, the variation (s.e. = $.754 \times 10^6 \mu^3$ per ml) is small compared to the temporal variation in the environmental concentration of particles in the south sector of Kaneohe Bay during the three months of this study (range: $5-17 \times 10^6 \mu^3$ per ml). The mean value of K ($2.68 \times 10^6 \mu^3$ per ml) can be helpful in deriving some general information about grazing in the bay.

Several experiments were attempted with animals and water from the middle sector of the bay, an area considered to be less eutrophic than the south sector (Caperon, et al. 1971). These experiments were unsuccessful, chiefly because the particle concentrations were so low that the factors discussed earlier, especially plant growth, had relatively greater effects than on south-sector experiments. However, the highest particle concentration observed in the middle sector was $2.16 \times 10^6 \mu^3$ per ml, below the average K for the south sector. Since the same types of grazers were found in the middle sector, there is an

indirect indication that grazing rates of the small grazers in the middle sector are not yet near their theoretical maximum.

Although Steinhilper (1970) concluded that particulate organic carbon in the south sector was well-mixed through the water column, on the two occasions when samples were taken from 0 and 5m for particle analysis in this study, the particle concentrations differed significantly ($p < .05$). The size distributions were similar to those in fig. 1, and these differences are therefore taken to be real differences in the concentration of grazers' food. Dr. S.A. Cattell's unpublished chlorophyll a data support this conclusion. Since the experimental water and animals were always taken from the surface, the results presented here do not necessarily apply to the entire water column; but the particle volumes at 5m in the south sector were 5.54 and 4.72 $\times 10^6 \mu^3$ per ml, both well in excess of the average K, and similar to the concentrations at which maximum grazing rates were observed in some of the experiments.

Another reason for caution in interpreting these experiments is the use of a mixture of species of grazers. Grazing rates here represent the behavior of a non-existent average animal in the collection, which may be a quantity of ecological interest, but may also be a misleading (and highly variable) one if the proportions of animals with different feeding patterns change from experiment to

experiment. Proportions were not recorded during counting of the animals, in order to minimize handling time, but the prepared suspensions were examined afterwards. The proportions of the species and stages varied somewhat, but there did not appear to be an association with any trends in the grazing rates. There is some precedent for the use of multi-species collections in investigations of feeding or uptake rates (MacIsaac and Dugdale 1967; Parsons, et al. 1969), and further refinement of such techniques may result in a valuable tool for the study of trophic level relationships.

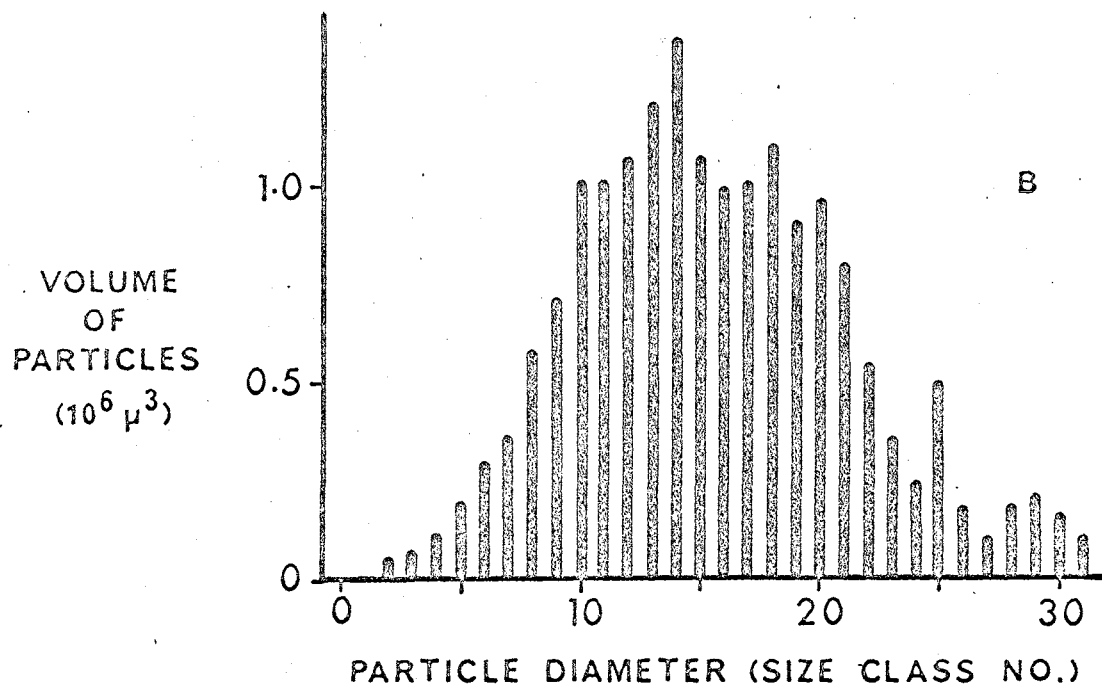
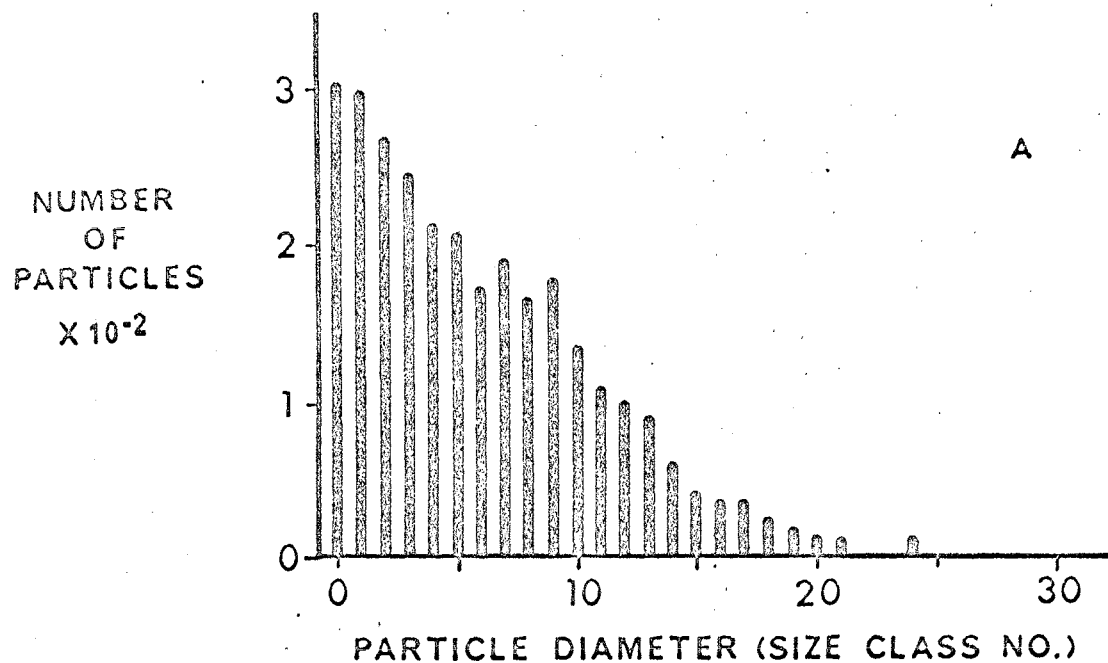


Figure 1. Size distribution of suspended particles by numbers (A) and by volume (B) in a 2ml sample from a control flask in the experiment on 8/3/71.
Diameter in microns = (size class no. \times 2.05) + 2.54.

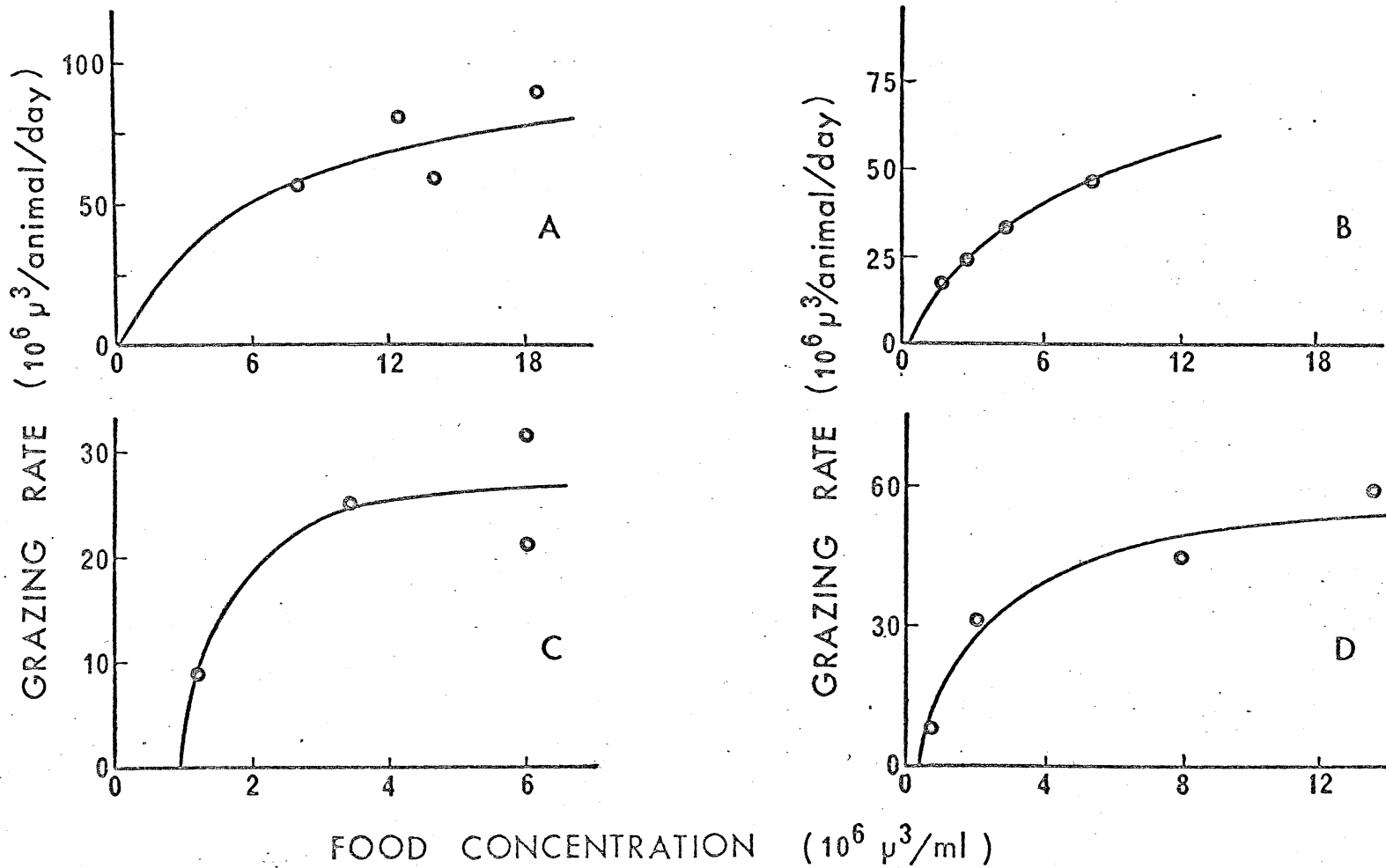


Figure 2. The relationship of grazing rate to food concentration for experiments with animals and water from the south sector of Kaneohe Bay. The curves are least-squares fits of equation (2) to the data.

Table 1. A comparison of the maximum grazing rates observed in the eight experiments with animals and water from the south sector of Kaneohe Bay with the maximum rates predicted by the hyperbolic model.

DATE 1971	OBSERVED MAXIMUM RATE ($10^6 \mu^3$ / animal/day)	PREDICTED MAXIMUM RATE (R) ($10^6 \mu^3$ / animal/day)	OBSERVED/ PREDICTED	K ($10^6 \mu^3$ /ml)
7/14	50.2	51.8	.969	2.53
7/17	101	98.6	1.02	2.55
7/28	30.7	24.6	1.25	1.12
7/30	47.8	47.2	1.01	1.34
8/3	46.1	87.7	.526	7.69
8/20	58.8	57.4	1.02	1.77
9/1	44.6	37.5	1.19	1.43
10/12	22.5	17.4	1.29	3.60
MEAN	50.2	52.8		2.68
STD. ERROR	8.30	10.0		.754

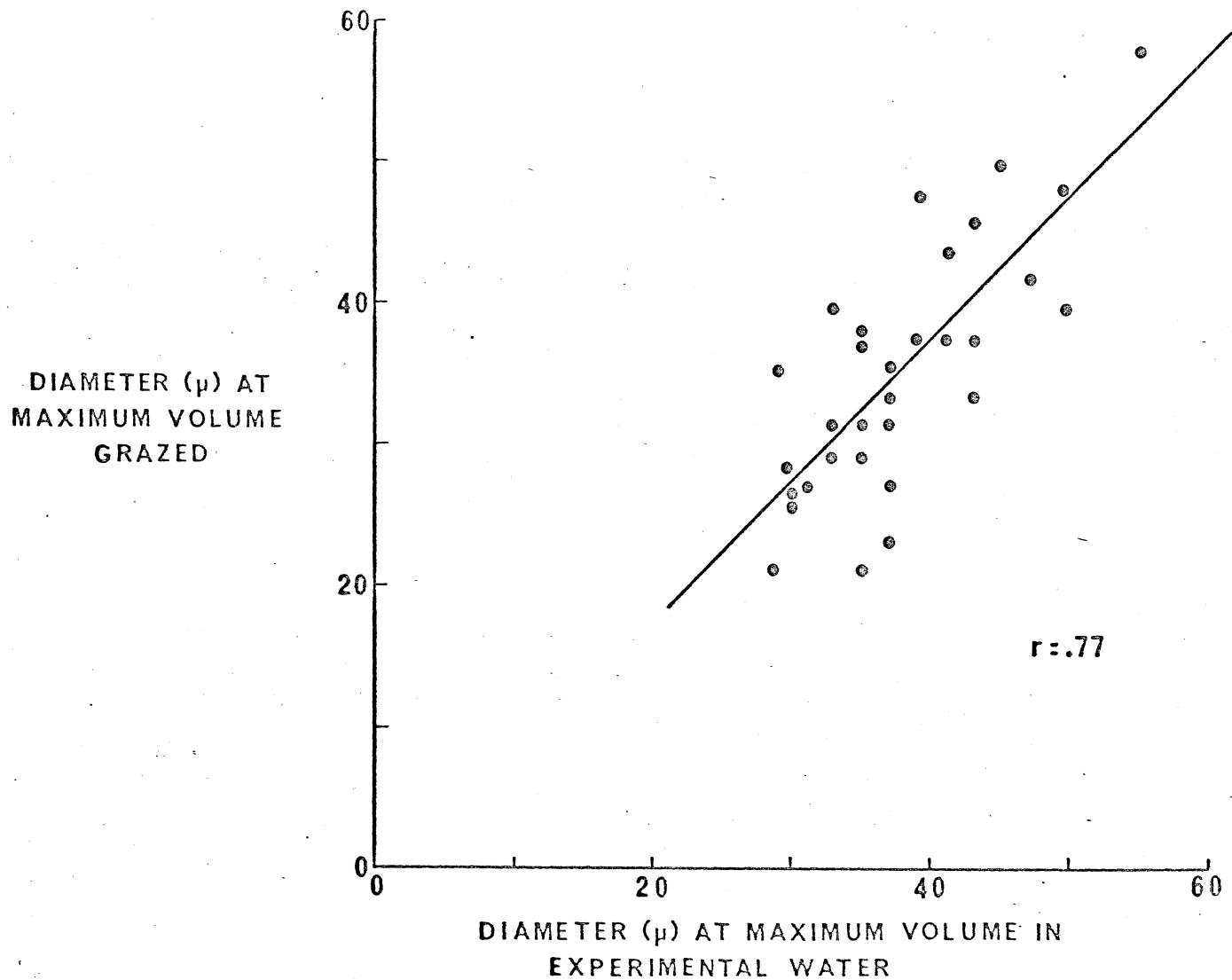


Figure 3. The relationship between the particle size most abundant in the water and the size grazed most heavily. Each point represents a single pair of flasks. The correlation is significant at the .001 level.

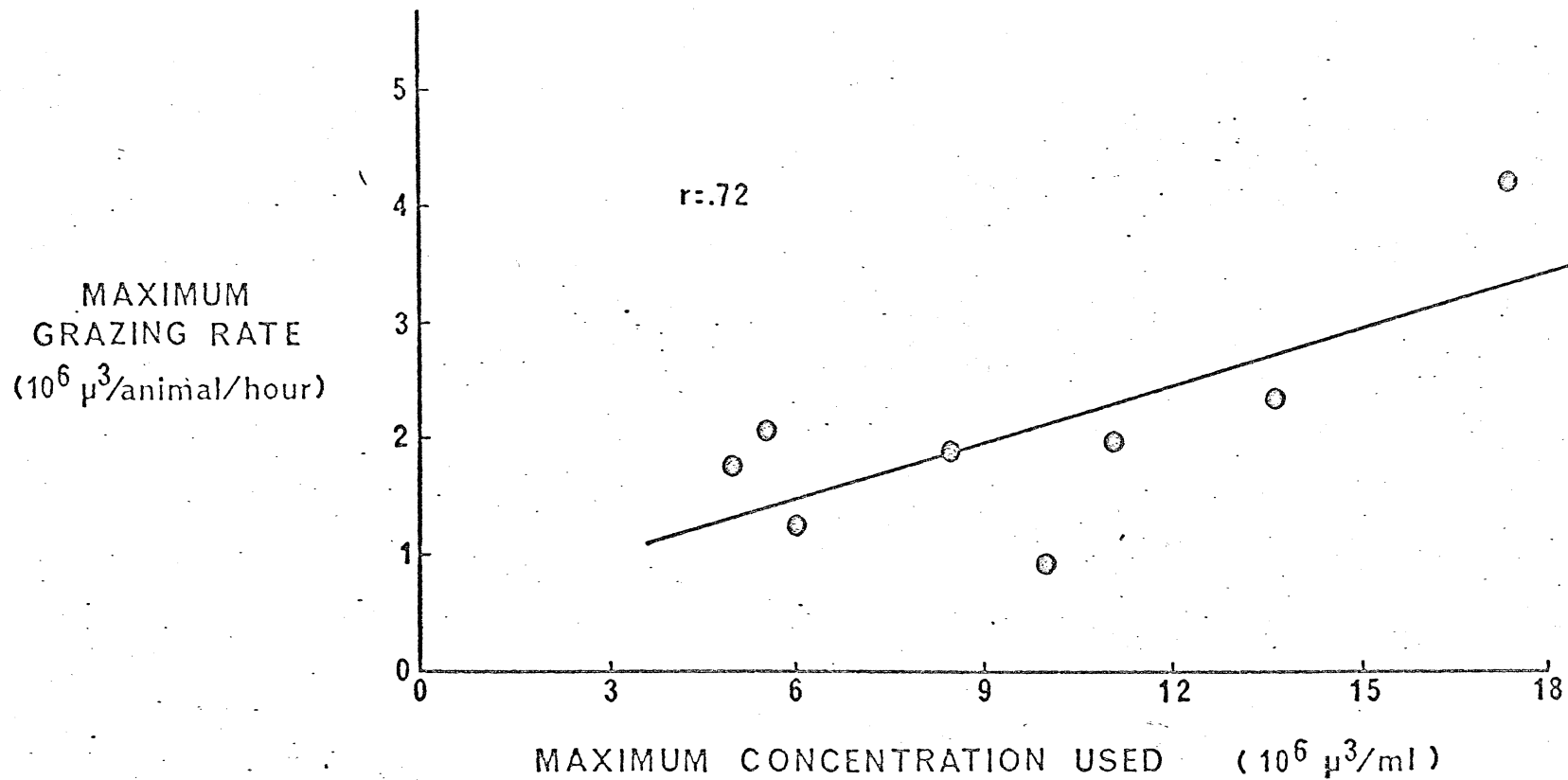


Figure 4. The relationship between the maximum food concentration in an experiment and the maximum observed grazing rate in the eight experiments with south-sector animals and water. The correlation is significant at the .05 level.

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APPENDIX

Table 2. Grazing rates at various food concentrations in the experiments with animals and water from the south sector of Kaneohe Bay.

DATE 1971	FOOD CONCENTRATION ($10^6 \mu\text{g/ml}$)	GRAZING RATE ($10^6 \mu\text{g/animal/day}$)
7/14	5.36	50.2
	5.22	31.7
	3.88	34.8
	2.90	28.3
7/17	17.4	101
	13.3	65.8
	11.1	88.3
	7.29	74.2
7/28	5.96	30.7
	5.98	19.7
	3.38	24.5
	1.09	8.93
7/30	11.1	40.3
	10.5	47.8
	6.48	36.0
	2.01	28.6
8/3	8.42	46.1
	4.56	31.9
	2.87	22.7
	1.80	14.8
8/20	13.6	58.8
	7.93	43.4
	1.95	31.0
	.678	8.52
9/1	4.98	42.0
	3.37	27.1
	2.51	44.6
	1.42	17.9
10/12	10.0	18.4
	7.33	17.3
	3.60	22.5