AN ABSTRACT OF THE THESIS OF

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(Name of student) (Degree)

in OCEANOGRAPHY presented on May 31 - 1973
(Major) (Date)

Title: FEEDING BEHAVIOR OF TWO POPULATIONS OF THE ESTUARINE COPEPOD ACARTIA CLAUSI

Abstract approved: Redacted for Privacy

The feeding behavior of adult females of two spatially separated populations of the Yaquina Bay, Oregon copepod Acartia clausi was studied. The copepod populations, one centered near the mouth and the other centered further up the estuary were distinguished on the basis of cephalothorax lengths, carbon and nitrogen content, and in vivo pigmentation differences. The down-bay females were longer, contained more carbon and nitrogen, and possessed fewer ventral pigment patches. Animals with intermediate cephalothorax lengths or other characteristics were not found.

Females collected during the spring and summer reproductive season of 1971 and 1972 were fed separately unialgal suspensions of the chain-forming, neritic, planktonic diatoms Thalassiosira nordenskioldii and Thalassiosira gravida over a range of diatom biomass concentrations. The volume of an average-sized

T. nordenskioldii particle was about half that of the average-sized T. gravida particle.

During the one-to-two year culturing period, the volume size class frequency distribution of both diatoms continuously shifted toward a higher frequency of smaller particles in non-grazed suspensions. The grazing activity of females from both populations of A. clausi also produced a higher frequency of smaller particles (single cells and two-cell chains) when fed T. gravida, but not when fed the smaller, shorter chained T. nordenskioldii. Suspensions of both diatoms were observed to be nearly free of cell fragments, with or without grazing. Observations made on feeding animals suggested that the scoop net activity of the maxillipeds and the clearance of particle accumulations on the setae of the feeding appendages might be the ways in which copepods dismembered long diatom chains into shorter chains and single cells.

Comparisons of ingestion rates obtained for each copepod population fed separately on both diatoms were made. The data showed that at low food concentrations, the ingestion rates of both populations' females fed T. nordenskioldii overlapped the ingestion rates of females fed T. gravida. At comparable higher food concentrations, T. gravida-fed females achieved higher ingestion rates than did T. nordenskioldii-fed animals. This trend was exhibited by both copepod populations. At very high food concentrations of T. nordenskioldii,

the ingestion rates of both populations appeared depressed.

Comparisons of ingestion rates for both populations fed T. nordenskioldii showed that the rates determined for the larger down-bay females were, on the whole, higher over the experimental range of food concentration than the ingestion rates determined for the smaller up-bay females. Ingestion rates determined for both up-bay and down-bay females fed the larger diatom, T. gravida, were nearly the same over the entire range of food concentration. These data suggested that the larger down-bay females could obtain greater amounts of food over a wider range of food particle size than could the smaller up-bay females. The up-bay animals perhaps could obtain more food biomass from T. gravida than from T. nordenskioldii, however, because they could break up the larger diatom particles into smaller ones. The down-bay animals also broke large T. gravida particles into smaller ones during grazing.

Specific ingestion rates were determined as amounts of carbon and nitrogen eaten per copepod carbon and nitrogen per day, expressed as percent. Animals ingested several times their body carbon and nitrogen content when fed either diatom. Specific ingestion rates were probably the result of several factors, including the "starved" condition of the copepods, and their reproductive state.

The Feeding Behavior of Two Populations of the Estuarine Copepod *Acartia clausi*

by

Harold Blane O'Connors, Jr.

A THESIS

submitted to

Oregon State University

in partial fulfillment of
the requirements for the
degree of

Doctor of Philosophy

June 1974

APPROVED:

Redacted for Privacy

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Date thesis is presented May 31, 1972

Typed by Opal Grossnicklaus for Harold Blane O'Connors, Jr.

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INTRODUCTION

Phytoplankton primary production, in the form of elements chemically configured as energy rich molecules, is trophically transferred in marine food webs. This fundamental process is initially facilitated by the process of grazing by small, planktonic crustaceans.

Much of the research that has centered upon lower trophic level processes in the marine environment has dealt with the planktonic crustacean grazers. Recent reviews of this work cover the bulk of what is known to date about zooplankton feeding behavior and nutrition (Sushchenya, 1963; Raymont, 1963; Cushing, 1964; Marshall and Orr, 1964; Jorgensen, 1966; Gauld, 1964, 1966; Conover, 1968; Frost, 1972). The majority of this reviewed research has been concerned with the larger oceanic crustaceans, especially the copepod genus *Calanus* and the euphausiids.

Trophic relationships of near-coast and estuarine microcrustaceans have received much less attention. However, as men increasingly depend upon coastal and estuarine environments for food and other resources, and increasingly use these environments as waste disposal sites (Ketchum, 1972), the need for increased understanding of the effect of these human activities on trophic processes involving

indigenous microcrustacean populations becomes urgent. Basic to this understanding is knowledge of the trophic processes themselves. Some effort towards illuminating some of these important biological processes has been accomplished.

Conover (1956) published one of the first treatises on small estuarine copepods not dealing exclusively with taxonomy or biomass distribution. In Long Island Sound, Conover found that temperature kept the two predominant species, Acartia clausi and A. tonsa, separated by season most of the time. When their distributions did overlap, competition, probably between the younger stages, eliminated the species less well adapted to prevailing conditions. In Conover's experiments, grazing was relatively inefficient when food organisms consisted of naked flagellates or other nannoplankton. In a similar vein, Parsons et al. (1967) found that differences in food availability to several grazing crustaceans could be attributed to the differences in the size and shape of the phytoplankton foods.

Gauld (1964) reviewed the literature on the feeding mechanisms of some planktonic copepods, including that on the estuarine genera Acartia, Temora, and Centropages. Two kinds of feeding mechanisms were described. The first mechanism, initially reported by Conover in Acartia, consists of a forward and ventral sweep of the spread first maxillipeds. The spread maxillipeds produce a kind of scoop net or seine (Marshall, 1949; Conover, 1956). The second

mechanism, first noted by Esterly (1916) and further described by Cannon (1928), is a filtering operation in which the first maxillipeds are held in such a manner so as to form an immobile filter or net. A current of water containing food particles is sucked through the net by the rotational exercise of the other cephalic appendages. This mechanism is typically observed in Calanus.

The feeding appendages described by Gauld appear to be more or less morphologically specialized for the type of feeding mechanism principally used. Gauld notes that many calanoid copepods use both mechanisms at different times for feeding on different foods, however.

Anraku and Omori (1963) investigated the relationship between the feeding habits and mouth part structure in some marine copepods, including the genera Acartia and Centropages, two estuarine forms. Acartia tonsa was found to be an omnivore that could efficiently ingest either plant or animal food particles. Centropages hamatus and C. typicus were omnivores, but both appeared to select animal food. A close relationship existed between mouth part morphology and food habits. The cephalic appendages of herbivorous species were found to be well developed for use in producing a pair of "feeding swirls," and to be well equipped to filter the particles thus brought to the mouth. Herbivore mandibles were provided with grinding dentition. In the case of predatory species, the feeding structures were on the whole simple, had fewer but stouter setae, and appeared to be for

prehensile use. The cutting edges of the mandibles had sharp tooth-like spines, likely for cutting prey. Feeding appendages of the omnivorous copepods studied appeared to be of an intermediate adaptation, with mandibular edges suitable for both cutting and grinding. Itoh's (1970) observations parallel and confirm those of Anraku and Omori (1963).

In addition to the population-dynamics approach of Conover (1956) and the morphological and functional-adaptation approach of Anraku and Omori (1963) and Itoh (1970), another fruitful point of view for trophic research has emerged from Ivlev's (1945) investigations of the feeding of planktivorous fishes. Ivlev proposed that a grazer's feeding intensity was proportional to the difference between the maximal ration capable of being taken in a given time and the actual ration. From this, Ivlev suggested that

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

where R and r correspond to the maximal and actual rations (ingestion rates), p is the concentration of food, and k is the coefficient peculiar to each system that defines the rate of change of r with changing p.

With zooplankton as the taxa of interest, several investigators have used Ivlev's model, with modifications, to examine the feeding behavior of some estuarine microcrustaceans (Parsons, LeBrasseur and Fulton, 1967; Parsons, LeBrasseur, Fulton, and Kennedy, 1969;

McAllister, 1970; Frost, 1972). Parsons et al. (1967, 1969) observed that ingestion occurred down to some low phytoplankton food concentration, then apparently ceased; i.e., grazing stopped before all cells had been removed from the water. Using Calanus as the grazer, McAllister (1970) was able to demonstrate an Ivlev-type relationship in laboratory feeding experiments. Frost (1972) fed adult Calanus females monospecific cultures of centric diatoms in laboratory experiments. He describes the relationship between ingestion rate and food concentration not in the hyperbolic form, but as a rectilinear response curve composed of two intersecting straight lines. Ingestion increases linearly with increasing phytoplankton food concentration up to a maximum rate that remains essentially unchanged with further increases in phytoplankton concentration. Frost refers to the phytoplankton concentration at which the maximum ingestion rate is first achieved as the critical concentration.

The objective of my research was to investigate the feeding behavior of adult females of two spatially and morphologically separated populations of the Yaquina Bay, Oregon copepod Acartia clausi that are fed two species of planktonic diatoms (Thalassiosira nordenskioldii and Thalassiosira gravida) that differ in size. I shall test the proposition that, under controlled conditions, the two copepod populations respond differently, in terms of their feeding behavior, to two different-sized diatom foods; i.e., different feeding niches

might be set up for the two copepod populations, even though the animals in each population are the same taxonomically and the two diatoms differ markedly only in size. The grazing responses would be expected to show quantitative differences because of grazer size alone, but modifications of food-density-dependent ingestion rates with time, because of changes in food size or palatability, are also anticipated.

Looking toward the future, this kind of feeding-behavior information might permit evaluation of the biological impact of uptake and accumulation of materials associated with the food, including toxic substances such as pesticides and heavy metals as well as basic nutrient elements. From this kind of information, planktonic herbivores with particularly sensitive feeding behavior response to the pollutant or element under study might be identified and possibly used as indicator organisms.

EXPERIMENTAL MATERIALS

Copepods

Adult females of two separate populations of the estuarine copepod Acartia clausi were used as grazers in my experiments. A. clausi was found by Steuer (1923) throughout the world's temperate oceans, but it is not commonly collected in other than near-shore areas and estuaries (Wilson, 1942). Zimmerman (1972) found that this species was the most common organism encountered in Clark-Bumpus samples from Yaquina Bay, Oregon, the estuary where I netted my animals (Figure 1). A. clausi totalled over 60% of all the animals Zimmerman collected. The population density of A. clausi is very low offshore (less than 10 animals m^{-3}), but rapidly increases upon entering the estuary and remains reasonably high throughout the length of the estuary. Concentrations over 3×10^4 animals m^{-3} have been recorded in late spring and early summer (Frolander et al., 1971; Zimmerman, 1972).

Zimmerman (1972) found an indigenous up-bay (buoys 29 and 39, Figure 1) population of A. clausi that did not appear to mix to any great extent with a down-bay population (bridge station, Figure 1) in Yaquina Bay. He concluded that both of these populations might have been derived from animals which overwintered in mid-bay (buoy 21, Figure 1). From December through March, the density of

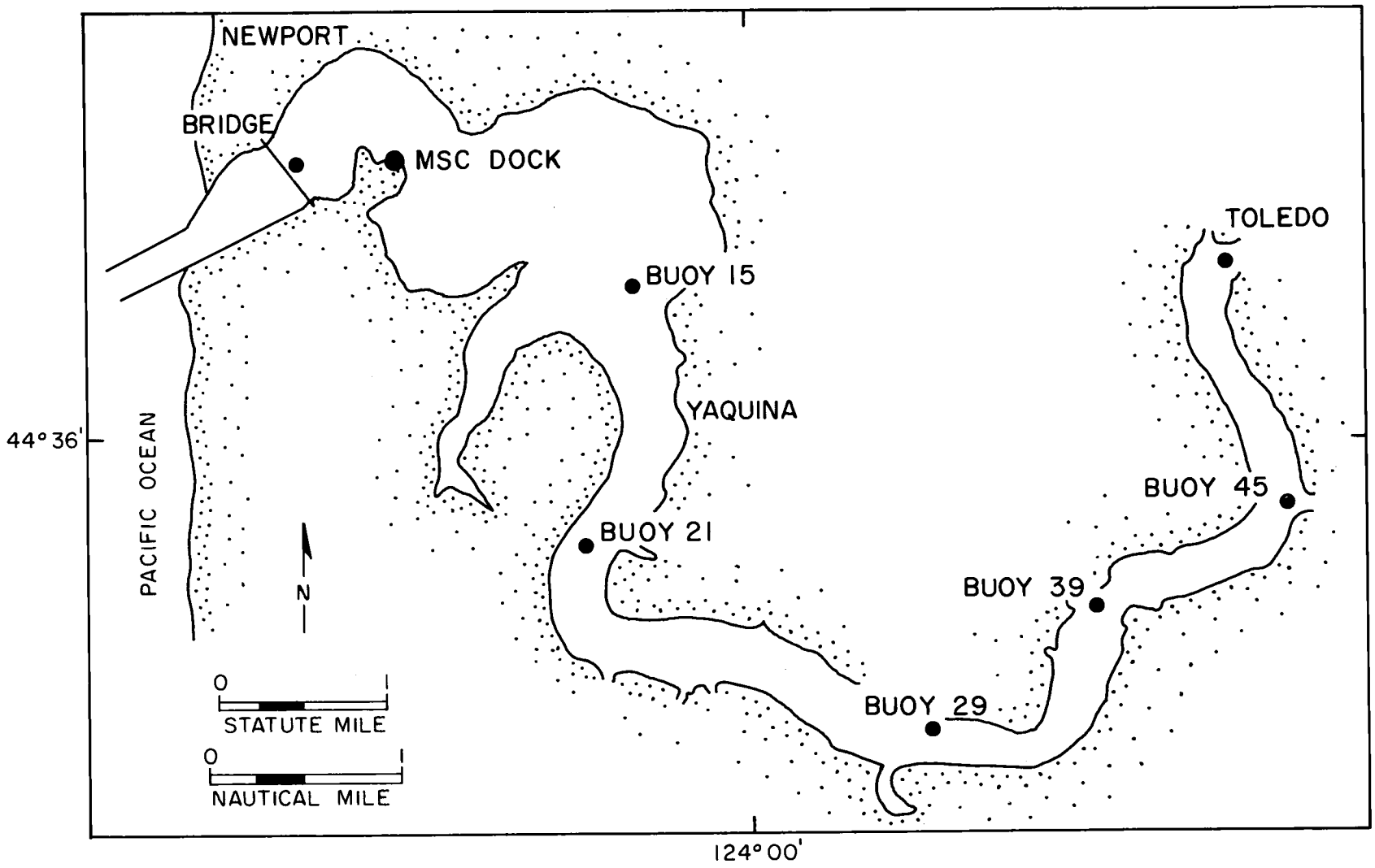

Figure 1. Map of Yaquina Bay showing stations.

A. clausi was highest at the mid-bay station while the density approached zero at the bridge station and at the furthest up-bay station (buoy 39). This trend was noted earlier by Frolander et al. (1971). A population center split was observed to occur during the spring and summer phytoplankton bloom, however (Zimmerman, 1972). The areas of highest abundance were progressively later up the bay and down the bay from buoy-21; thus, the overwintering buoy-21 population appeared to have given rise to the subsequent bridge and buoy-39 populations.

As the bay warmed in spring, Zimmerman (1972) and I independently found that the up-bay female A. clausi decreased in size relative to the down-bay females (Figure 2a). The lack of a size gradient up the bay is perhaps indicative of genetic polymorphism within the original population. These up-bay and down-bay animals could also be distinguished on the basis of their carbon content (Figure 2b) and nitrogen content (Figure 2c). In terms of morphology, the up-bay females were not only smaller, but their cephalothoraxes were more spindle-shaped. In vivo the animals were more densely covered over their length with dark, rust-colored ventral patches. The down-bay females were larger and had more pear-shaped cephalothoraxes. They had fewer, but larger, bluish ventral patches (in vivo), concentrated anteriorly. The swimming legs of living down-bay females had a bluish cast which was absent in the living

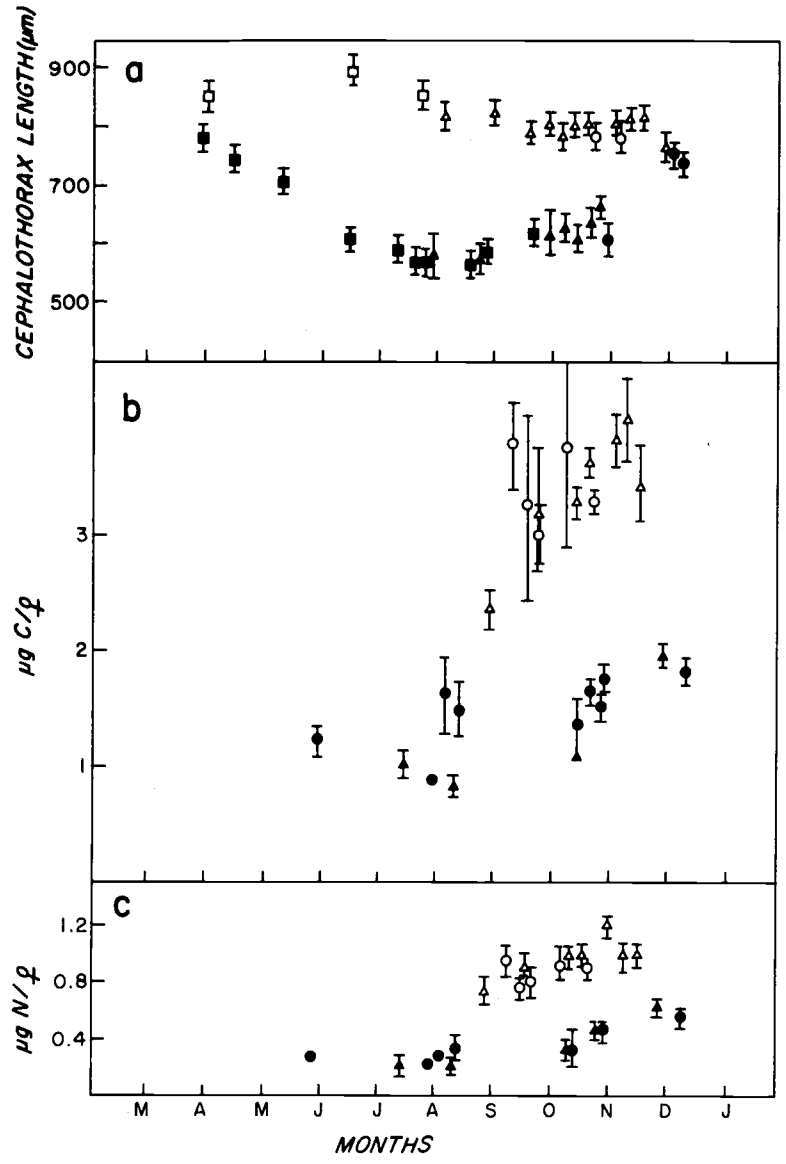

Figure 2. a, cephalothorax lengths; b, micrograms of carbon per female; and c, micrograms of nitrogen per female determined for Acartia clausi females collected at two stations in Yaquina Bay.

Date	Bridge station (down-bay females)	Buoy 21 to 39 station (up-bay females)
1969	□	■
1971	○	●
1972	△	▲

The 1969 cephalothorax lengths data were redrawn from Zimmerman (1972). Data points with bars equalling one standard deviation are means of n = 3 to 15 measurements.

up-bay females.

Water

Water used in my experiments was collected as needed in 50-liter polyethylene carboys at the Yaquina Bay bridge (Figure 1). During my summer collection period, surface and bottom salinities at the bridge varied between 31‰ and 34‰. At the same time, the bottom water salinity at buoy 21 (in the up-bay collecting area) varied between 28‰ and 34‰ (Zimmerman, 1972; Flynn, unpublished data). Animals were predominantly collected in the salt wedge up-bay, with very unproductive collections in the relatively fresh, near-surface water.

Phytoplankton food species

Monospecific suspensions of the planktonic, chain-forming, neritic diatoms Thalassiosira nordenskioldii Cleve and T. gravida Cleve (Cupp, 1943) were used as food for the female A. clausi grazers. These clones were originally isolated from a water sample obtained from Auke Bay, Alaska in the summer of 1971. Chains of Thalasiosira also were observed in phytoplankton netted from Yaquina Bay. The two Thalassiosira species had similar shapes: short cylindrical cells that formed the basic chain unit, with each cell separated from the two adjacent cells in the chain by a thin filament. T. nordenskioldii

cells were, on the average, only about half the volume of T. gravida cells, and occurred in shorter chains.

The diatoms were grown in a modified Guillard and Ryther (1962) enriched sea water medium. This medium is equivalent to 1/3 strength Basic Medium "Ala" used for culturing algae at Oregon State University (Curl, unpublished), with NH_4Cl added. The diatoms grew well in this medium under low-intensity fluorescent light provided by two General Electric F40 WWX Deluxe Cool White® tubes set about 45 cm above the 2 or 2.8 l glass flasks containing the cultures. The cultures were maintained and grown in a 12.5° C Controlled Environments® chamber.

METHODS AND TECHNIQUES

Down-bay zooplankton were netted in oblique tows between the bridge station and the Oregon State University Marine Science Center dock (Figure 1), while up-bay animals were taken in a similar manner between the buoy-21 station and the buoy-39 station (Figure 1). Collections were made weekly Thursday morning (0830 to 0900) from 8 July to 9 December, 1971, and from 22 June to 30 November 1972.

The net contents were placed in thermally insulated jugs which had been partially filled with bay water just collected at the bridge station. The collections were immediately transported to the Corvallis campus of Oregon State University. Here they were held in 3.5 l glass jugs after being diluted to at least twice their original zooplankton concentration with previously collected filtered and cooled bridge water.

Storage was in a Controlled Environments® chamber set for 12.5° C. Surface and bottom water temperatures at the bridge were 10 ± 3° C, while the bottom water temperature at the buoy-21 station was 12.5 ± 3° C (Zimmerman, 1972; Flynn, unpublished data). The collections were stored in dim light and kept no longer than Thursday afternoon through Friday afternoon to provide A. clausi for the experiments.

A number of adult females were pipetted into 200 ml of membrane-filtered, 12.5° C bay water in 250 ml beakers. Groups of

12, 18, or (usually) 24 animals were separated and held for 18 to 24 hours in dim light in the chamber before being fed. Animals were separated Thursday afternoon for experiments begun Friday morning, and separated Friday morning for experiments begun Saturday morning.

The biomass concentration of the diatom food suspensions was varied among experiments. Experiments were begun by gently pouring the 200 ml of filtered water with the food-deprived animals into a 900 ml beaker into which had just been poured 700 ml of a diatom food suspension. The total 900 ml mixture yielded the desired phytoplankton food biomass concentration. This mixture was placed in dim light in the chamber, and the females were allowed to graze for 24 hours. The suspension was kept well-mixed by pouring it gently back and forth between the original 900 ml beaker and a similar beaker every 4 to 8 hours. The grazed volumes were inspected for dead animals when mixed and at the end of the grazing period. In the majority of experiments, no dead animals were found at any time. On several occasions, one dead animal was detected during the grazing period. In several experiments, one or two dead females were found at the end of the grazing period. Copepod mortality considered constant and low in all experiments; therefore, no mortality corrections were applied to the data.

The diatom cultures used as food were always log-phase batch

cultures which had been diluted 1/4 to 1/2 with culture medium at the time the experiments were begun. This dilution was done to provide an excess of NH_4^+-N so as to eliminate any possibility that zooplankton excretion could increase the diatom growth in the grazed volume as compared to that in control volume.

Controls were identical volumes of the diatom suspension without copepods. They were prepared and maintained for each experimental food concentration in exactly the same manner as were the grazed volumes.

The biomass and particle numbers of phytoplankton, plus size class frequency distributions were determined for the control suspensions at the beginning of each experiment, and were determined for both the controls and grazed suspensions at the end of the 24-hour grazing period. A model B Coulter® electronic particle counter was used for all determinations. A number of comprehensive descriptions of the Coulter principle, instrumentation, and applications are available (Matten, Brackett and Olson, 1957; Maloney, Donovan and Robinson, 1962; El-Sayed and Lee, 1963; Sheldon and Parsons, 1967b; Strickland and Parsons, 1968), but a brief account of my specific techniques with the instrument are given here. In the Coulter® counter, particles suspended in an electrolyte are caused to flow through a small aperture. The aperture has been supplied with immersible electrodes, one on each side of the aperture, to

produce an electric field. The passage of a particle through the electric field causes a short-duration voltage pulse, linearly proportional to the particle's volume. It is these pulses that permit the counting and sizing of the particles flowing through the aperture. A 200 μm-diameter aperture was used throughout my experiments.

Calibration of the aperture was achieved using a nearly monosized particle, ragweed pollen. A few ml of an alcohol dispersion of the pollen was added to Millipore® -filtered bay water, and the suspension was vigorously agitated to produce a uniform distribution of single pollen grains. The mean diameter of the pollen grains was 20.3 μm, a value calculated from 200 pollen grain diameters measured microscopically with a calibrated ocular micrometer. The calibration was checked at least weekly during the experiments. Electolyte salinities and suspension temperatures were well within the instrument's specifications for linear responses to increments in counts and particle size.

The response of the instrument to the passage of particles through the aperture is limited to a small zone of sensitivity around the aperture (Matten, Brackett and Olson, 1957). Even so, there is a possibility that two or more particles will pass through the aperture coincidentally, thus giving a single voltage pulse which would (1) incorrectly lower the count, and (2) give incorrect particle volume information. To take into account both types of coincidence errors,

I determined experimentally the particle concentration at which coincidence passages began when counting the two diatoms using the 200 μm-diameter aperture. To estimate this particle concentration, counts were made, inclusive of all particle size classes occurring in a 2 ml sample, of successive dilutions of a concentrated suspension of each of the diatoms. These counts were then plotted against percent dilution and a line was fit by inspection through the points (Figure 3). The particle concentration at which this line departed from the linear relationship generated by the data points for the lower particle concentration was designated as the concentration at which coincidence events had begun to lower the observed count below the actual particle concentration. The critical concentration was about 7000 particles $2\ ml^{-1}$ for T. nordenskioldii (Figure 3a), and (with two separate determinations shown) about 8000 particles $2\ ml^{-1}$ for T. gravida (Figure 3b). All particle counting and sizing in these experiments was below those coincidence threshold concentrations. Dilutions of the suspensions were made when necessary.

The biomass concentration ($\mu m^3\ 2\ ml^{-1}$) and particle concentration (particles $2\ ml^{-1}$) were determined in a 2 ml sample of the controls and the grazed suspensions of the two diatoms by scanning the size class distributions with a window four threshold units wide; thus, the number of particles $2\ ml^{-1}$ in each of the size classes (windows), from the smallest diatom particle to the largest, was counted. The

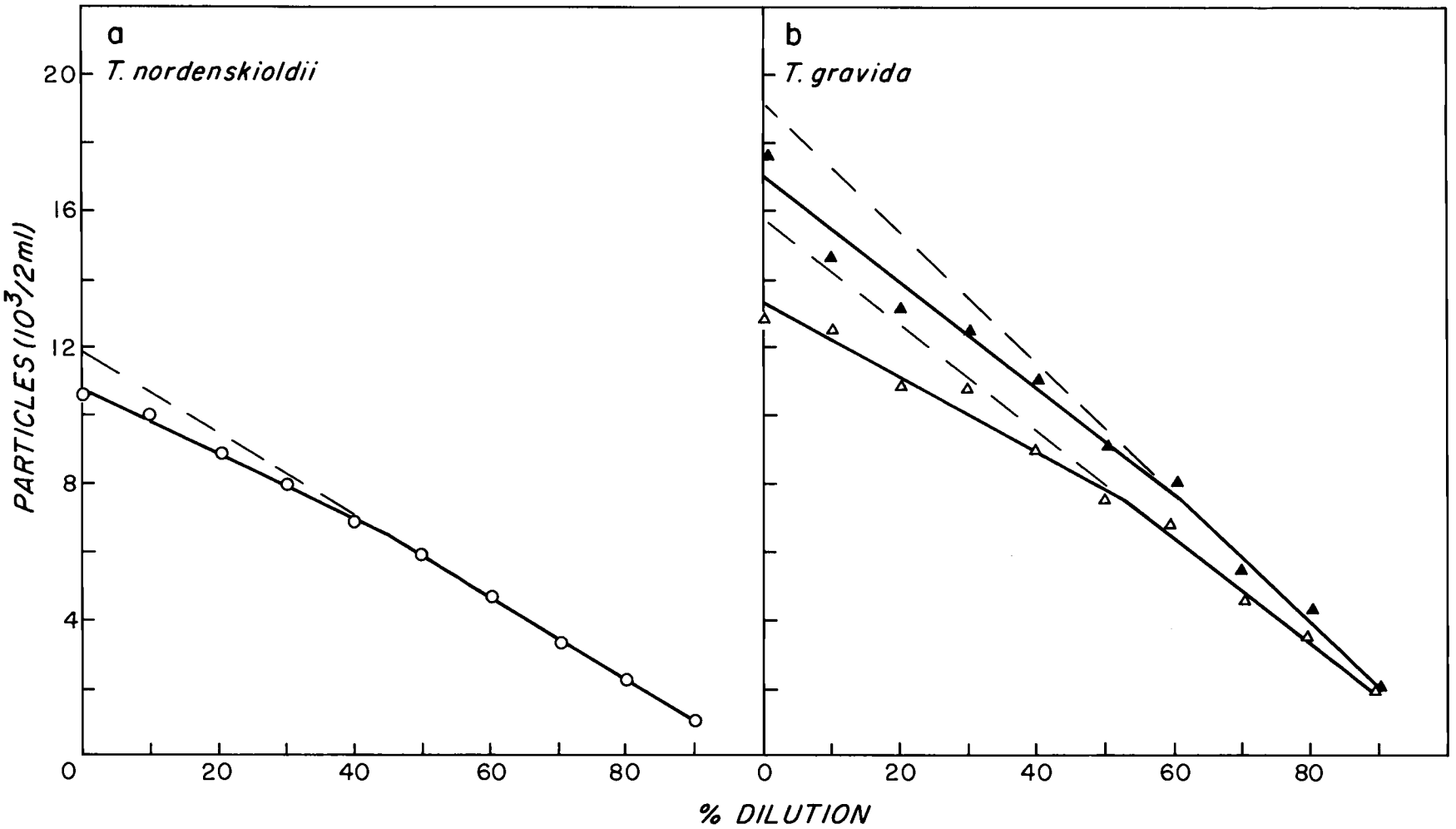

Figure 3. Estimates of the particle concentration at which coincidence events began for 200 μm diameter aperture. Data points are means of three particle counts of T. nordenskioldii a or T. gravida b.

total concentration of particles was calculated as the sum of the particles $2\,ml^{-1}$ in all the size classes. The biomass concentration was calculated as the number of particles in a given size class multiplied by the mean volume in that size class, with these products summed for all the size classes. Three replicated estimates were done. The precision of some of these estimates is shown in Figure 4. Two standard deviations (as a percent of the mean for the three replicate determinations) were calculated and plotted against (1) the biomass concentration ($10^6\ \mu m^3\ 2\,ml^{-1}$) and (2) the particle concentration (10^3 particles $2\,ml^{-1}$) for both T. nordenskioldii and T. gravida. While the counting and sizing error due to coincidence events increased with increasing particle concentration above a threshold value, the precision of the estimates of biomass concentration and particle concentration decreased with decreasing particle concentration. Therefore, dilutions were often necessary to optimize the particle concentration from the standpoints of both coincidence events and counting precision.

As a check on volume sizing in the electronic particle counter, diatom particles of both species were measured microscopically, using a calibrated ocular micrometer, several times during these experiments. In these measurements, I noted the number of diatom cells making up each particle, as well as the diameter and length of each of these cells in the particles. Using these observations, I

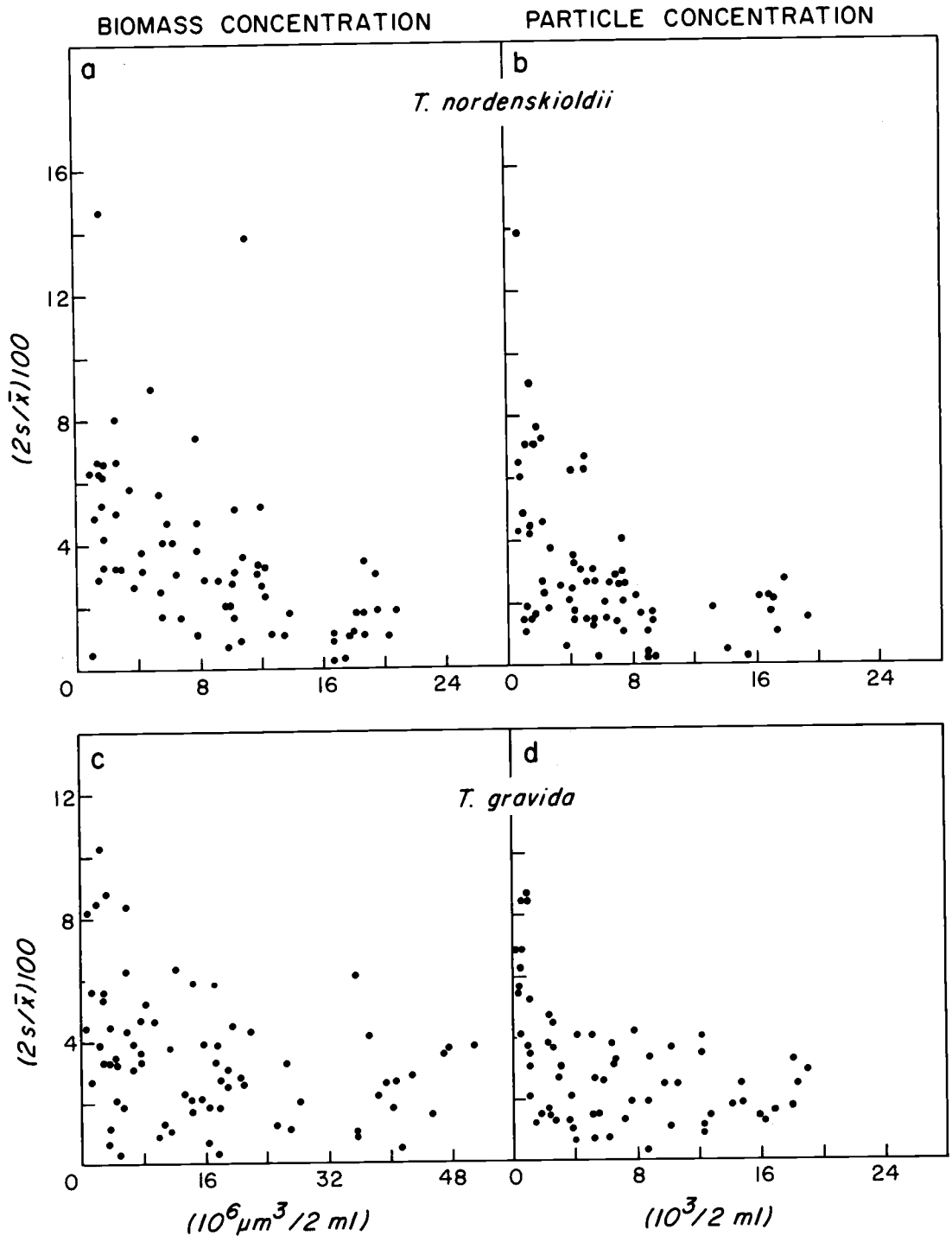

Figure 4. Precision of Coulter® counter estimates of diatom biomass concentration (a and c) and particle concentration (b and d) for T. nordenskioldii and T. gravida. Note abscissa scale change in c.

calculated the volume of the particles, assuming the cells were cylinders, by summing the individual cell volumes observed in each particle. These observations were made so as to include representative particles, from the smallest to the largest, that occurred in the diatom suspensions over the experimental period.

From the phytoplankton biomass estimates determined initially for the control and determined again after 24 hours for the control and grazed phytoplankton suspensions, the grazing rates ($I = \mu m^3$ of phytoplankton eaten female^{-1} 24 hours^{-1}) were calculated in a manner similar to that used by Parsons, LeBrasseur and Fulton (1967) and Strickland and Parsons (1967). The equation was:

$$I = [(C_{24} - G) \times \frac{V}{n}] \cdot \qquad (2)$$

where C_{24} is the estimate of the diatom biomass concentration (μm^3 ml^{-1}) in the control volume, and G is the estimate of diatom biomass concentration (μm^3 ml^{-1}) in the grazed volume, both determined 24 hours after the experiment had begun. V is the volume of the grazed suspension (900 ml) and n is the number of females placed in the grazed suspension. The quantities C_{24} and G were means of three replicate determinations.

Although the means of the three replicate determinations of G were always less than the means of the three replicated determinations of C_{24}, I thought it necessary, because of variability in the data

(Figure 3), to test the difference statistically. Using Student's "t" distribution, and assuming that $\sigma^2_{C_{24}} = \sigma^2_G$ and that both populations of data were normally the null hypothesis, $H : C_{24} = G$ was rejected at the .05 level of significance.

The average phytoplankton concentration in the grazed suspension, $\bar{P}$ (in μm^3 ml^{-1}), was calculated by:

$$\bar{P} = [\frac{Co + G}{2}]. \quad (3)$$

Where Co is the diatom biomass concentration in the control at the beginning of the experiment, G is the diatom biomass concentration in the grazed suspension at the end of the experiment per ml. The quantities Co and G (in terms of particles ml^{-1}) were also used in equation 3 to give $\bar{P}$ in terms of particles ml^{-1}.

Values for $\bar{P}$ computed using equation 3 were within 1% of $\bar{P}$ values estimated for representative experiments using the commonly seen equation

$$\bar{P}_t = P_0 e^{(k-g)t}, \quad (4)$$

where $\bar{P}_t$ is the phytoplankton biomass concentration when t = the mid-time of the experiment (12 hrs.), k is the instantaneous rate of phytoplankton increase, g is the instantaneous rate of grazing mortality, and P_0 is the phytoplankton biomass concentration in the control and grazed suspension at the beginning of the experiment. The close agreement between the linear and exponential calculations occurred

because grazing removal of cell biomass was small relative to initial cell concentrations, over the 24-hour grazing period.

Plots of the number of particles ml^{-1} in the volume size classes making up the size class distribution for each of food diatoms were used to determine (1) the impact of copepod grazing upon the diatom volume size class frequency distributions on a particle basis and (2) volume size class frequency distributional shifts that were observed during the phytoplankton culturing and were therefore not related to the grazing activity.

A number of unfed females were dried for about 24 hours at 50-60° C on GF/A glass fiber filters, then were stored over anhydrous calcium chloride for later analysis of carbon and nitrogen content. Volumes of T. nordenskioldii and T. gravida suspensions of known biomass concentration were also filtered onto small GF/A glass fiber filters, then dried at 50-60° C for about 24 hours. These samples also were later analyzed for their carbon and nitrogen content using a Carlo-Erba® CHNO elemental analyzer. These data were used to compute carbon and nitrogen linear regression equations so that ingestion rates could be converted to their C and N equivalents. Such conversions might help to illuminate how well the copepods are able to fulfill their needs for these nutritionally critical elements, a feature that would not be revealed by the ingestion relationships expressed in terms of diatom volumes only.

RESULTS

Particle-volume size class distribution in the absence of grazing

The volume size class frequency distributions of T. gravida or T. nordenskioldii were determined as a matter of course in each experiment for the initial control volume at the beginning of each experiment, for the post-24-hour contol, and for the grazed volumes at the end of each 24-hour experiment. Representative data from both the electronic particle counter determinations and particle volume calculations (based on ocular micrometer measurements) are given for T. nordenskioldii (Figure 5) and for T. gravida (Figure 6). The data points in each volume size class indicates the particle count range for the three replicate particle concentration estimates. The curves were fit by inspection. The T. nordenskioldii volume size class frequency distribution a (Figure 5) was obtained in August 1971, early in the experiments, while distribution b was obtained near the end of the work, in October 1972. A shift in the mode to a smaller volume size class is noted, from the volume size class having a mean volume of 1430 μm^3 to one having a mean volume of 990 μm^3.

The mean particle volume (the T. nordenskioldii biomass concentration divided by the particle concentration) has also shifted, from 1836 μm^3 to 1171 μm^3. The chronology of this shift is shown in Figure 5c. The increase in mean particle volume observed in

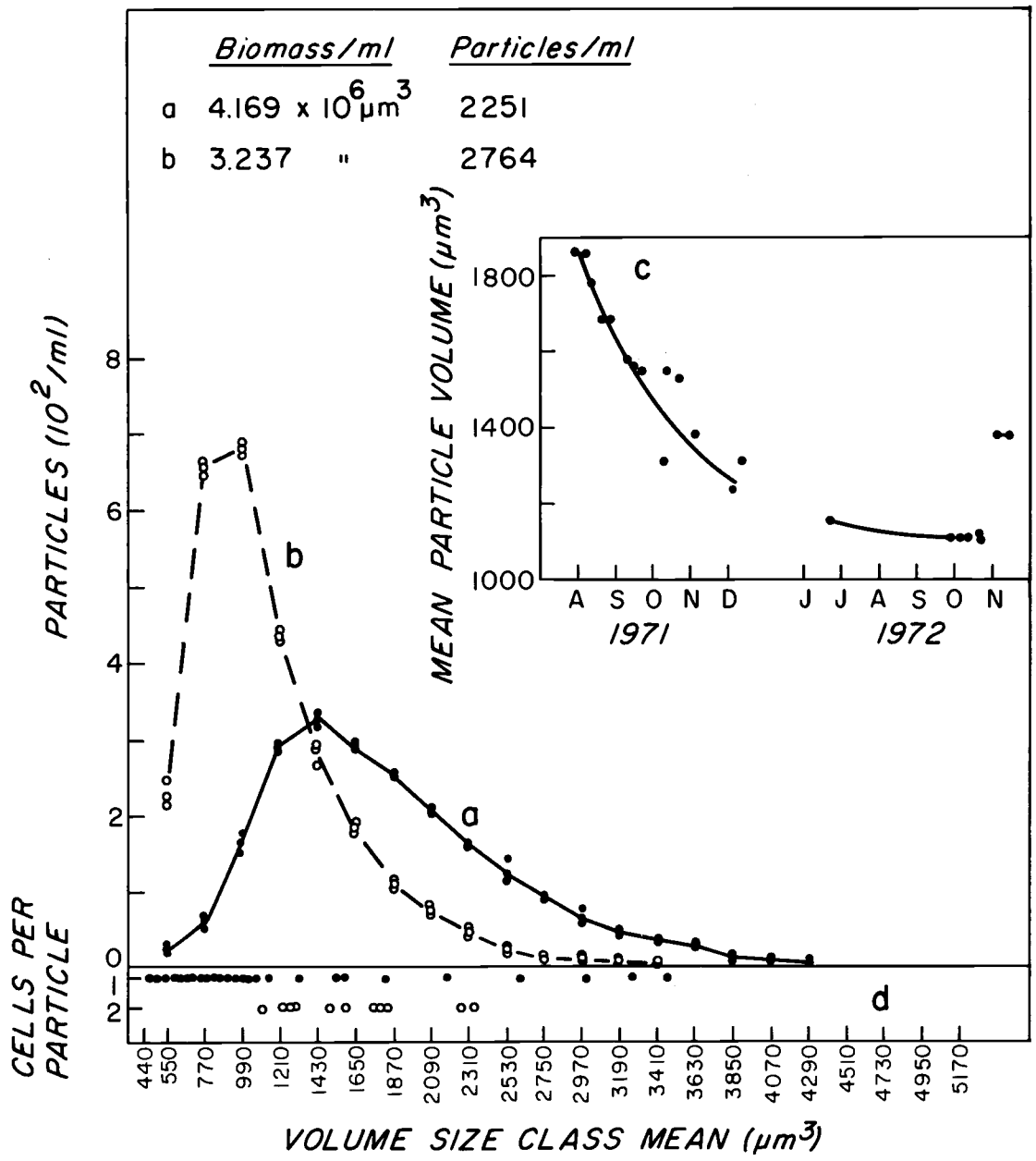

Figure 5. a and b, volume size frequency distributions for T. nordenskioldii. Distribution a was determined in August 1971. Distribution b was determined in October 1972. c, chronology of the mean particle volume change. d, volume size range for T. nordenskioldii particles. The data points in each size class represent three replicate particle concentration estimates.

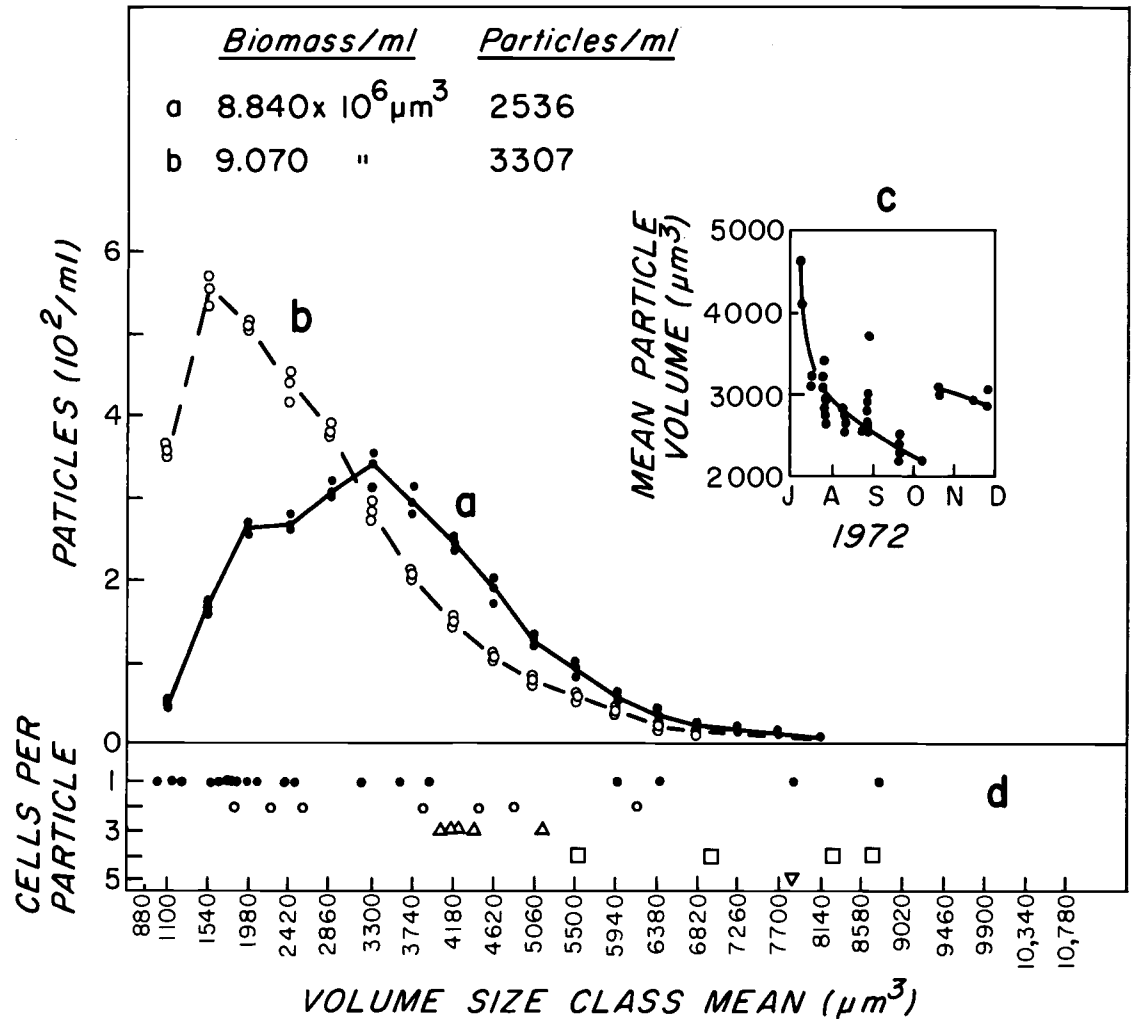

Figure 6. a and b, volume size class frequency distributions for T. gravida. Distribution a was determined in mid-July 1972. Distribution b was determined in mid-September 1972. c, chronology of the mean particle volume change. d, volume size range for T. gravida particles. The data points in each size class represent three replicate particle concentration estimates.

November 1972 was due to the introduction of a different subclone of the original T. nordenskioldii clone at that time. This introduction was necessitated by a growth chamber power failure early in October 1972, which killed the subclone then in use. The new subclone, used in the last few experiments, had been cultured at a higher temperature (about 14° C) under a different light and nutrient regime.

Figure 5d shows the volume size range of T. nordenskioldii particles in terms of single-celled particles and chains of two cells as they occurred during the experiments. The number of cells and volume of the particles was determined from ocular micrometer measurements. These measurements were not made during the earliest experiments; however, microscopic examination during these early experiments revealed that chains of three and four cells did occur. When ocular micrometer measurements were begun in August 1971, the longest chain observed contained two cells. Single cells were numerous. The two-cell chains were composed of cells about 550 μm^3 to 1200 μm^3 in volume connected by a thin filament. The total volume of these chains ranged between 1100 μm^3 to 2300 μm^3. Volumes of single cells ranged between 473 μm^3 and 1100 μm^3. Some of the larger cells appeared to be in the process of division.

Single particles larger than about 1100 μm^3 (Figure 5d) were composed of single-cell units that were attached valve to valve (without an interconnecting filament) to form a sausage-like particle. The

volume of these particles was as large as 3472 μm^3. As the culturing continued, single-celled particles of less than 1100 μm^3 formed the bulk of the particles present (Figure 4b). The Coulter® counter was adjusted to count particles with volumes greater than 440 μm^3 but less than 5880 μm^3, a volume range that included the total particle volume range determined from the ocular micrometer. T. nordenskioldii, then, provided tha A. clausi females with a particulate food suspension having a particle volume size range of 473 μm^3 to 3400 μm^3. Within this range, the majority of the diatom particles were single cells or 2- to 4-celled chains, with the proportion of single-celled particles increasing during the experimental period.

The T. gravida volume size class frequency distribution a was obtained in mid-July 1972, soon after the culturing of this diatom had begun (Figure 6). Distribution b was observed in mid-September 1972, well into the experimental period. As in Figure 5 for T. nordenskioldii, the distributional mode and the average particle volume had shifted. The mode was in the volume size class having an average volume of 3300 μm^3 in distribution a, while about two months later, the mode had shifted to a volume size class with an average volume of 1540 μm^3. During the same period the mean particle size had also shifted, from 3487 μm^3 in distribution a to 2743 μm^3 in distribution b.

The chronology of the mean particle volume shift is summarized in Figure 6c. The increase in mean particle volume observed in

October 1972 was due to the introduction into the experiments of a different subclone of T. gravida which had been maintained at 12.5°C under low light and with a less frequent nutrient input.

Ocular micrometer measurements begun early in the work showed that T. gravida particles were composed of one to five cells (Figure 6d). One-celled particles ranged in volume from 990 μm^3 to about 2600 μm^3. Single particles larger than about 2600 μm^3 were composed of attached single cell units forming a sausage-shaped particle, the volume of which was as large as 8800 μm^3. Attachment of the single cell units was valve to valve, without an interconnecting filament. The other multiple-celled particles were composed of cells connected by a thin filament. The individual cells in these chains ranged in volume from 990 μm^3 to about 3400 μm^3. Some of the larger cells appeared to be in the process of division. Two-celled chains ranged in volume from about 1900 μm^3 to 6200 μm^3, three-celled chains ranged from about 4200 μm^3 to about 5100 μm^3, four-celled chains from about 5500 μm^3 to 8700 μm^3, and the very rare five-celled chains were about 7800 μm^3 in volume.

With time in culture, the majority of the particles were single-celled and chains of two cells, as determined by both electronic particle counter data and microscopic examination (Figure 6b). Only a very few three- and four-celled particles were observed microscopically.

The Coulter® counter was adjusted to count particles which had a volume of greater than 880 μm^3 but less than 11000 μm^3, a volume range that included all of the particle volume range determined from the ocular micrometer measurements made during the experiments.

T. gravida, then, provided the A. clausi females with a particle volume range of 990 μm^3 to 8800 μm^3, slightly greater than twice the particle volume range made available by T. nordenskioldii. Within this range the diatom particles were single-celled and two- to four-celled chains, with the proportion of single-celled and double-celled particles increasing during the experimental period.

Particle-volume size class distribution in the presence of grazing

Volume size class frequency distributions for the initial control, the post-24 hour control, and the grazed suspensions have been plotted for two experiments for each diatom in order to examine the impact of the A. clausi females upon the particle volume size class frequency distribution (Figures 7 and 8). The bars through the data points indicate the particle count range in that volume size class. The curves were fit by inspection.

The distributions labelled 1 in Figures 7a, 7b, and 7c are for the initial control, the post-24-hour control and grazed suspensions respectively, in an experiment begun 5 August 1971 (before the T. nordenskioldii volume size class frequency distribution had shifted

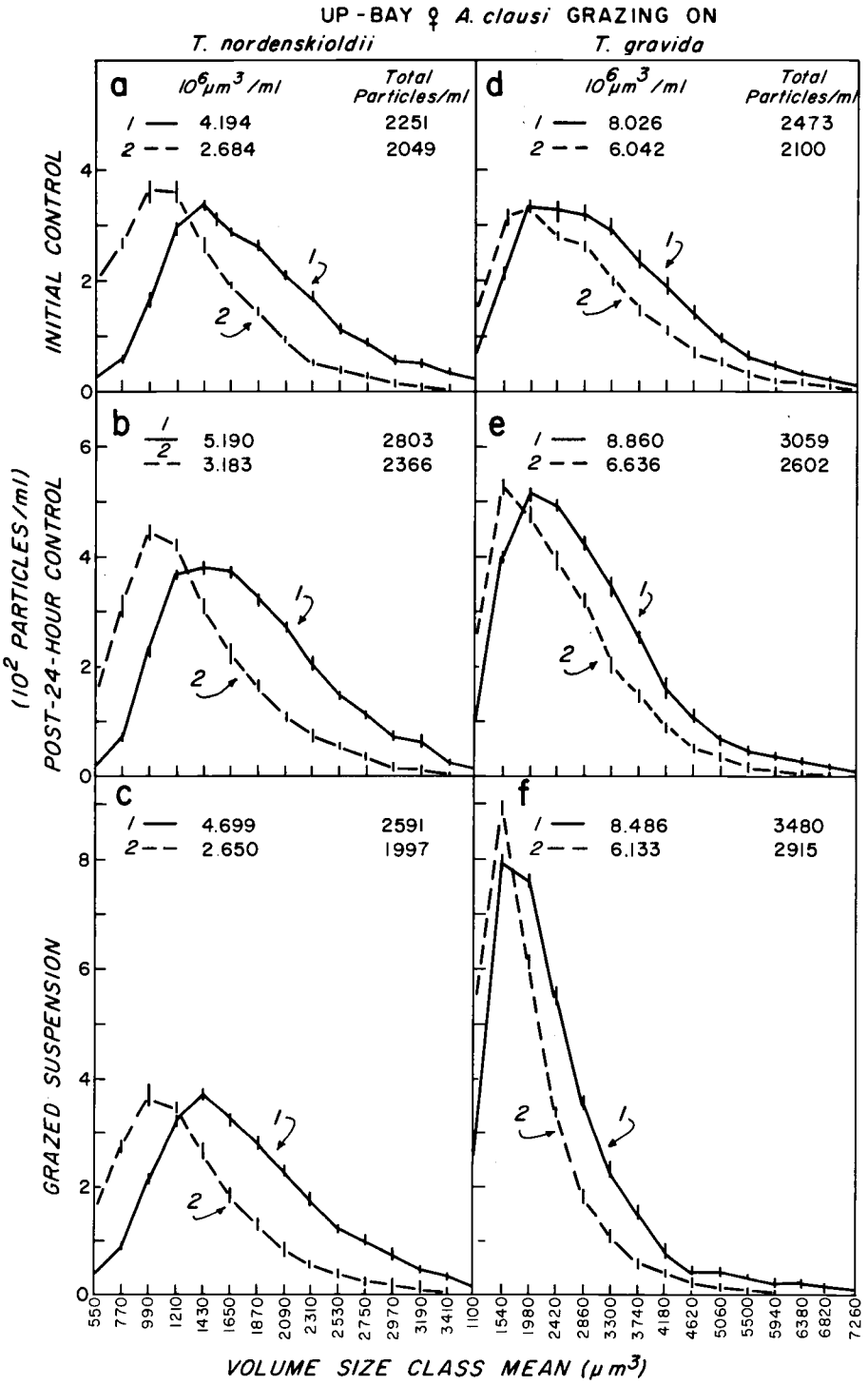

Figure 7. Volume size class frequency distributions for T. nordenskioldii and T. gravida for initial controls (a and d), post-24-hour controls (b and e), and up-bay female A. clausi grazed suspensions (c and f). See text for details. Bars indicate the range of the particle counts.

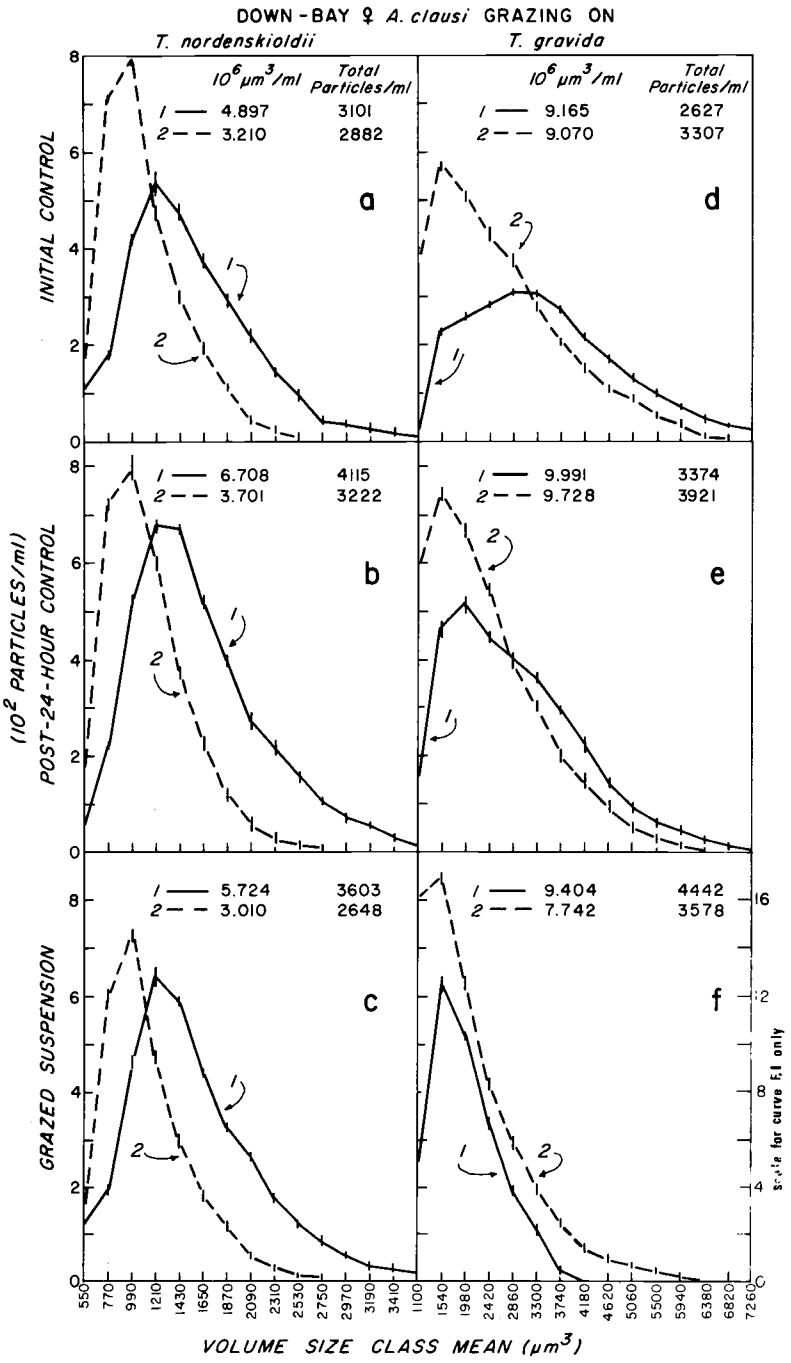

Figure 8. Volume size class frequency distributions for T. nordenskioldii and T. gravida for initial controls (a and d), post-24-hour-controls (b and e), and down-bay female A. clausi grazed suspensions (c and f). Note particle concentration scale change for f1. See text for details. Bars indicate the range of the particle counts.

in culturing). The curves labelled 2 in Figure 7a, 7b, and 7c are the volume size class frequency distributions for the initial control, the post-24 hour-control and the grazed suspension, respectively, for an experiment run 9 December 1971, after the T. nordenskioldii volume size class frequency distribution had shifted. Inspection of these distributions demonstrate that the grazing activity of the up-bay A. clausi population females had little impact on the volume size class distributions. For the 5 August experiment, the mean particle volume in the post-24-hour control was 1852 μm^3, while in the corresponding grazed suspension the mean particle volume was 1812 μm^3, less than a 3% change. A very similar pattern occurred in the 9 December 1971 experiment. The mean particle volume in the post-24-hour control was 1344 μm^3, versus 1327 μm^3 in the grazed suspension.

The same kinds of plots for T. gravida experiments show a markedly different result. Distributions labelled 1 in figures 7d, 7e, and 7f are volume size class frequency distributions for the initial, the post-24-hour control and the grazed suspensions, respectively, for an experiment begun 13 July 1972. This experiment was performed before the particle volume distribution shift was much in evidence. Distributions labelled 2 are the same measurements for an experiment performed 10 August 1972, after much of the volume size class frequency distribution shift in the T. gravida culture had occurred. Comparisons of the data for post-24-hour controls and

the grazed suspensions reveal the marked impact of the up-bay female A. clausi grazing activity. For the 13 July 1972 experiments, the average particle volume in the post-24-hour control was 3246 μm^3, as compared to 2438 μm^3 in the grazed suspension. A similar pattern occurred in the 10 August 1972 experiment. The average particle volume in the post-24-hour control was 2550 μm^3, compared to 2104 μm^3 in the grazed suspension.

In both of the experiments involving T. gravida, grazing activities caused the volume size class distribution of the diatom to be skewed, with greater frequency of smaller particles. Microscopic examination of the grazed suspensions revealed no abundance of cell fragments or detritus; in fact, all diatom suspensions used in all of the experiments (including those with T. nordenskioldii) were nearly free of non-diatom particles before and after the 24-hour grazing periods. The grazed T. gravida suspensions did have a higher frequency of single cells than the non-grazed 24-hour control suspensions, however, strongly suggesting that the copepods were breaking up chains into smaller units as a result of their feeding activity.

In Figure 8, I have plotted volume size class frequency distributions for the initial control, the post-24-hour control, and the grazed diatom suspension for two experiments for each diatom, in order to examine the impact of the down-bay female A. clausi grazing activity upon the food particles. As before, distributions labelled 1 in

Figures 8a, 8b and 8c were observed in an experiment near the beginning of the work (11 September 1971) before the T. nordenskioldii had greatly changed in volume size class frequency distribution. Distributions labelled 2 were obtained on 28 September 1972 over a year after the beginning of the T. nordenskioldii culturing and after the volume size class frequency distribution of the culture had shifted toward smaller size classes.

Inspection of the curves for T. nordenskioldii (Figures 8a, 8b, and 8c) demonstrated that the grazing activity of the down-bay A. clausi females had little impact on the volume size class frequency distribution of the grazed suspensions. This result was similar to that observed with the up-bay females. For the 11 September 1971 data, the mean particle volume in the post-24-hour control was 1630 μm^3, while in the corresponding grazed suspension it was 1589 μm^3, less than a 3% change. In the 28 September 1972 experiment, the average particle volume in the post-24-hour control was 1148 μm^3, versus 1137 μm^3 in the grazed suspension.

Again the T. gravida experiments showed markedly different results. For the 3 August 1972 experiment (distributions labelled 1 in Figures 8d, 8e, and 8f) which was begun before the T. gravida volume distribution had shifted, the average particle volume in the post-24-hour control was 2971 μm^3. In the corresponding grazed suspension it was 2117 μm^3. The 21 September 1972 experiment

(distributions labelled 2 in Figures 8d, 8e, and 8f) which was begun after the volume distribution shift, gave similar results. Mean particle volumes were 2481 μm^3 in the post-24-hour control and 2164 μm^3 in the grazed suspension. Grazing impact by both up-bay and down-bay females on T. gravida was thus very pronounced, whereas there was no measureable impact by either copepod or T. nordenskioldii distributions.

Ingestion by up-bay females

The ingestion rate, I, in units of μm^3 of diatoms eaten female^{-1} 24 hours^{-1}, was determined in experiments carried out over a range of diatom biomass concentration for the up-bay and down-bay females feeding separately on each of the food diatoms in unialgal suspensions.

Ingestion rates of up-bay animals were plotted against $\overline{P}$, in terms of μm^3 ml^{-1} (Figure 9a), and against $\overline{P}$, in terms of diatom particles ml^{-1} (Figure 9b). Envelopes were drawn by inspection in order to include all the ingestion rate measurements made on each diatom. While the variability of the data contained within each envelope is large, the difference between the two envelopes is larger, over much of the experimental range of $\overline{P}$. The ingestion rate envelope for T. nordenskioldii totally overlays the T. gravida envelope over a range of $\overline{P}$ from about 0.5×10^6 to about 3×10^6 μm^3 ml^{-1} (Figure 9a), but separation of the envelopes begins above 3×10^6 μm^3 ml^{-1}. The

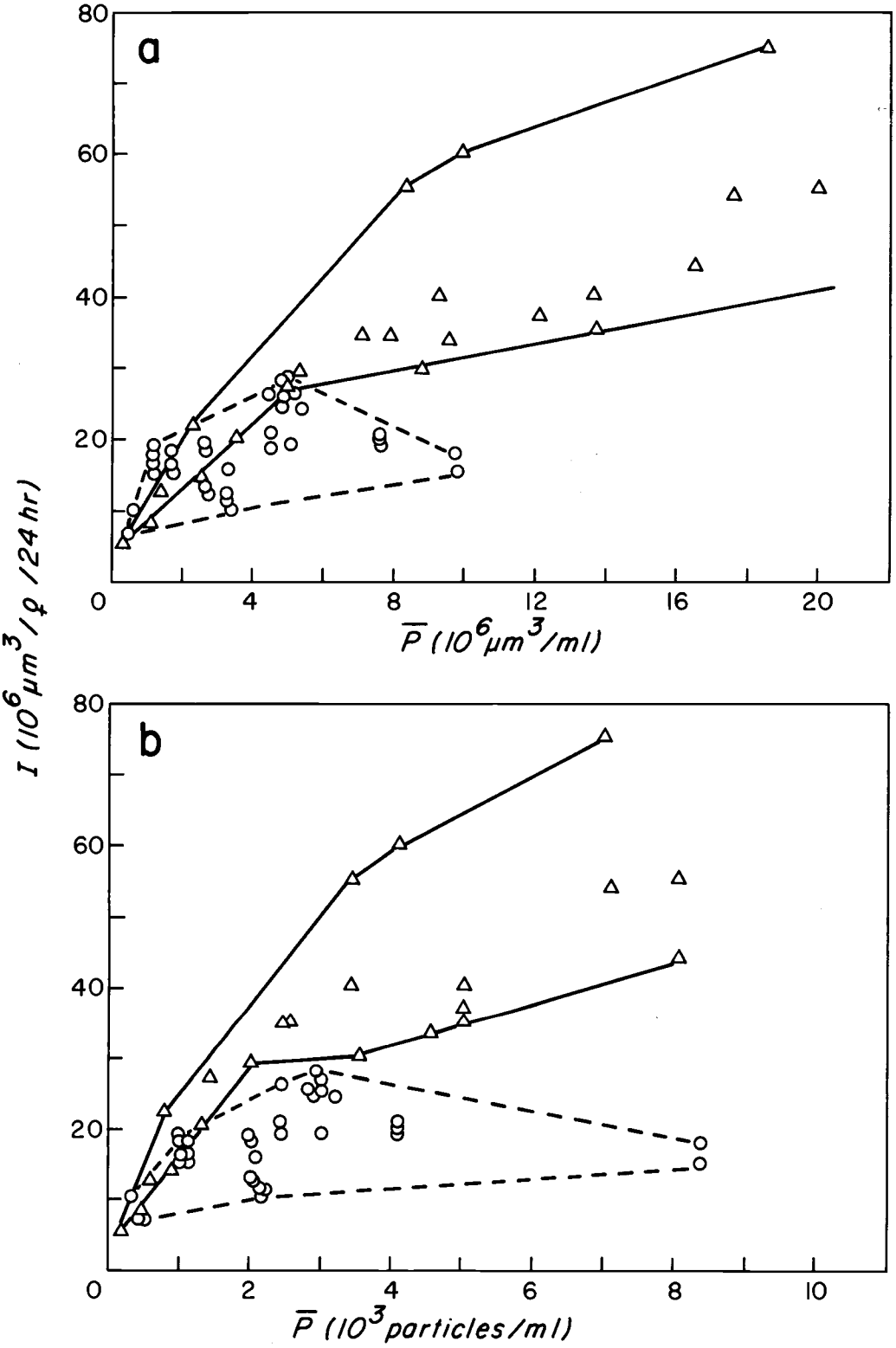

Figure 9. Ingestion rate envelopes for up-bay A. clausi females grazing on T. nordenskioldii, O and T. gravida, Δ. a, $\bar{P}$ in terms of $\mu m^3 ml^{-1}$. b, $\bar{P}$ in terms of particles ml^{-1}. Dashed line encloses T. nordenskioldii ingestion rates, solid line encloses T. gravida ingestion rates.

T. nordenskioldii experiments were carried out to a mean biomass concentration of approximately 9.5×10^6 μm^3 ml^{-1}, which corresponded to a very concentrated, rapidly growing culture. Similarly, the maximum biomass concentration of T. gravida (about 20×10^6 μm^3 ml^{-1}) represented a dense, vigorous culture.

When the ingestion rate data for the up-bay animals are plotted against mean particle concentration rather than mean volume concentration, it becomes obvious that the overlay of the two envelopes is reduced at lower particle concentrations (Figure 9b). Even though the range of $\bar{P}$ for T. nordenskioldii was only about half that of T. gravida in terms of μm^3 ml^{-1} (Figure 9a), the range of $\bar{P}$ as particles ml^{-1} is nearly equal for both diatoms (Figure 9b). This feature is the result of smaller particles making up the volume size class distribution for T. nordenskioldii (see Figures 5 and 6).

Ingestion by down-bay (bridge) females

The copepod ingestion rate envelope determined for both diatoms, when plotted against $\bar{P}$ in terms of μm^3 ml^{-1}, overlapped nearly completely below an average biomass concentration of approximately 5×10^6 μm^3 ml^{-1} (Figure 10a). Variability in the data made exact interpretation impossible, however. At T. gravida concentrations above about 5×10^6 μm^3 ml^{-1}, the ingestion rate envelope remains broad throughout the remaining range of $\bar{P}$. Ingestion rates of females

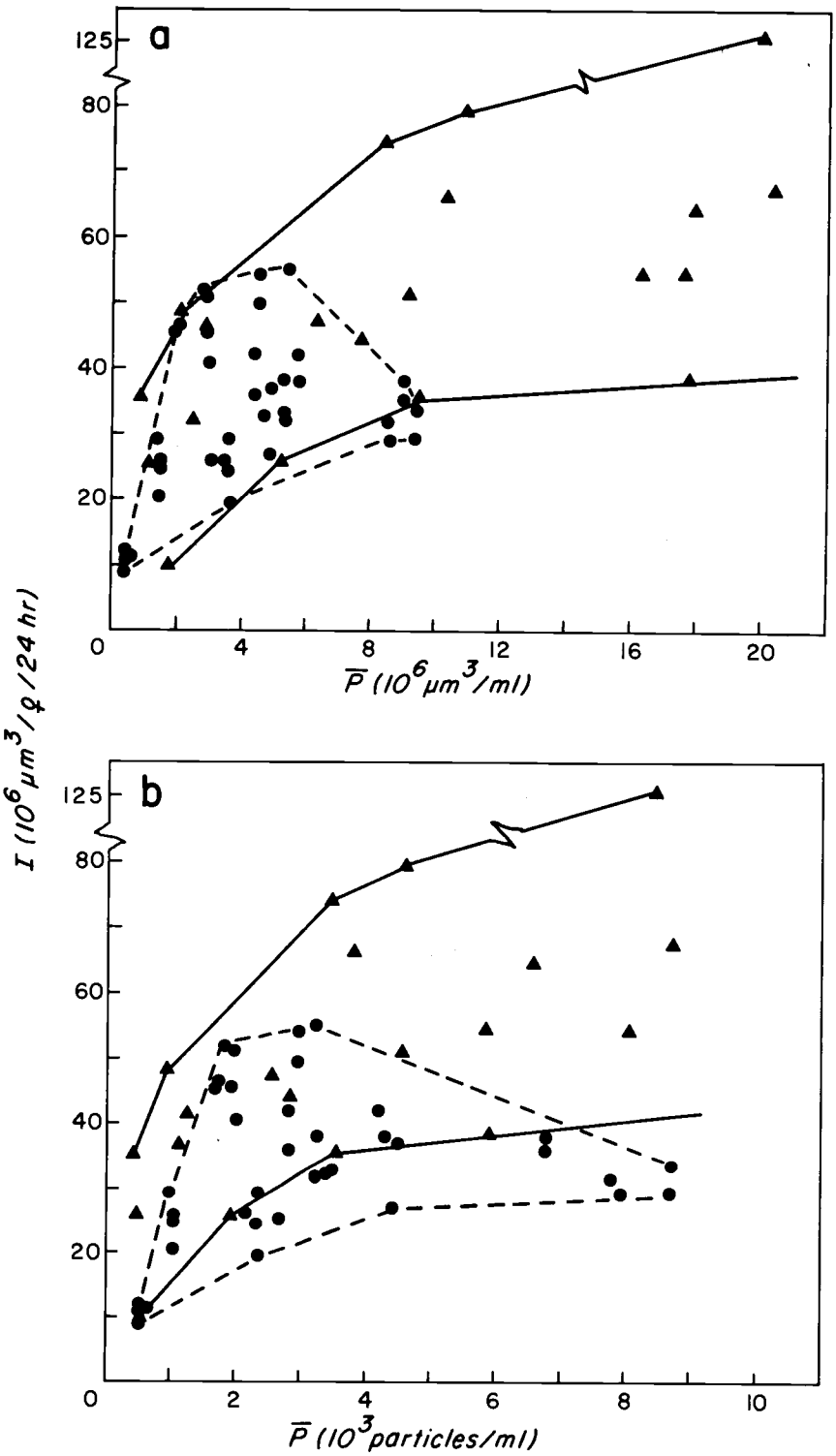

Figure 10. Ingestion rate envelopes for down-bay A. clausi females grazing on T. nordenskioldii, ● and T. gravida, ▲. a, $\bar{P}$ in terms of $\mu m^3 ml^{-1}$. b, $\bar{P}$ in terms of particles ml^{-1}. Dashed line encloses T. nordenskioldii ingestion rates, solid lines encloses T. gravida ingestion rates.

fed T. nordenskioldii apparently decline at food concentrations greater than about 5×10^6 μm^3 ml^{-1}.

Similar results were obtained when the ingestion rates were graphed against $\bar{P}$ in units of particles ml^{-1} (Figure 10b). Considerable envelope overlap was again apparent below an average particle concentration of about 4×10^3 particles ml^{-1}. Above the $\bar{P}$ value, the T. gravida envelope continued to increase but that of T. nordenskioldii declined.

Comparison of ingestion rate envelopes

In Figure 11, I have compared the ingestion envelopes for both the up-bay and down-bay female A. clausi determined in experiments with T. nordenskioldii as the food organism. Figure 11a shows the ingestion rates as a function of $\bar{P}$ in units of μm^3 ml^{-1}, while Figure 11b shows the same ingestion rates plotted against $\bar{P}$ as particles ml^{-1}. In both sets of envelopes the ingestion rates of the down-bay females, on the whole, are higher at any given $\bar{P}$ than the ingestion rates of the up-bay females. Within the range of $\bar{P}$ from about 0.5 to about 6×10^6 μm^3 ml^{-1} (Figure 11a) and from about 500 to about 4000 particles ml^{-1} (Figure 11b), the lower ingestion rates determined for the down-bay animals overlap the upper half of the range of ingestion rates determined for the up-bay animals. Both the up-bay and down-bay animals appear to achieve their maximum ingestion

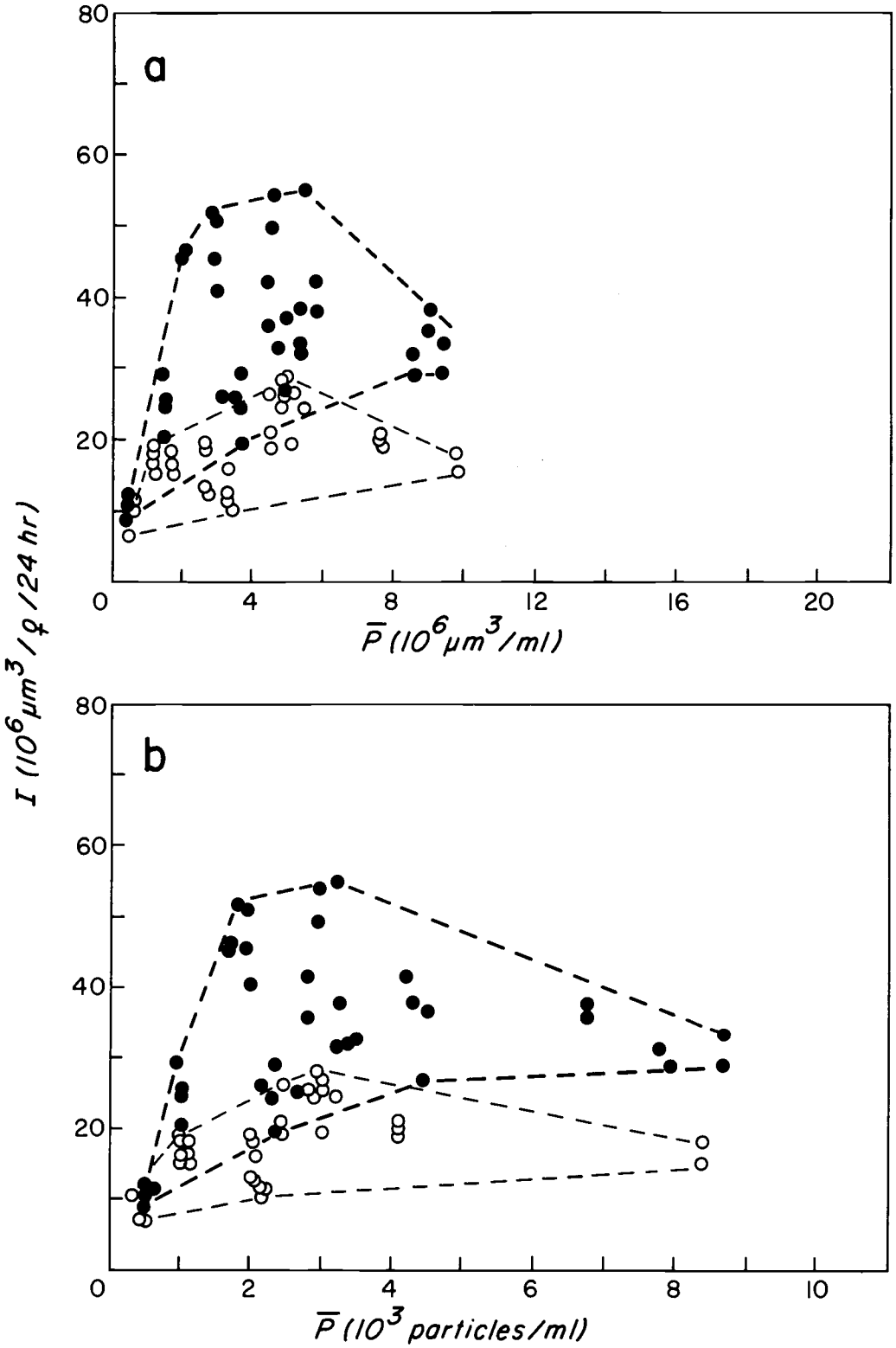

Figure 11. Comparison of T. nordenskioldii ingestion rate envelopes for up-bay, O and down-bay, ● A. clausi females. a, $\bar{P}$ in terms of μm^3 ml^{-1}. b, $\bar{P}$ in terms of particles ml^{-1}. light dashed line encloses ingestion rates for up-bay females, heavy dashed line encloses ingestion rates for down-bay females.

rates at $\bar{P}$ values of about 5×10^6 μm^3 ml^{-1} or 3×10^3 particles ml^{-1}. Above these diatom concentrations, ingestion rates tended to decline. Ingestion rate variability, particularly with down-bay females, tended to be the greatest at particle volume and number concentrations associated with maximum ingestion rates.

In Figure 12, I have graphed the ingestion rates for both the up-bay and down-bay copepods feeding on T. gravida. While the ingestion rates of the down-bay females tended to exceed those determined for the up-bay females, there was no separation of the envelopes over the entire experimental range of $\bar{P}$. The ingestion rates determined for the up-bay females appeared to be closely coupled to increasing $\bar{P}$ below a $\bar{P}$ value of 7×10^6 μm^3 ml^{-1} (Figure 12a) or 3×10^3 particles ml^{-1} (Figure 12b). Above these values the data were scattered. No clear relationship existed for the down-bay animals over the complete range of $\bar{P}$, although trends of increasing I with increasing $\bar{P}$ are obvious.

Specific ingestion rates for the two populations of A. clausi females

Measured volumes of rapidly growing T. nordenskioldii and T. gravida suspensions of known biomass concentration, the range of which was comparable to the range of $\bar{P}$ in the experiments, were filtered and dried on glass fiber filters for carbon and nitrogen

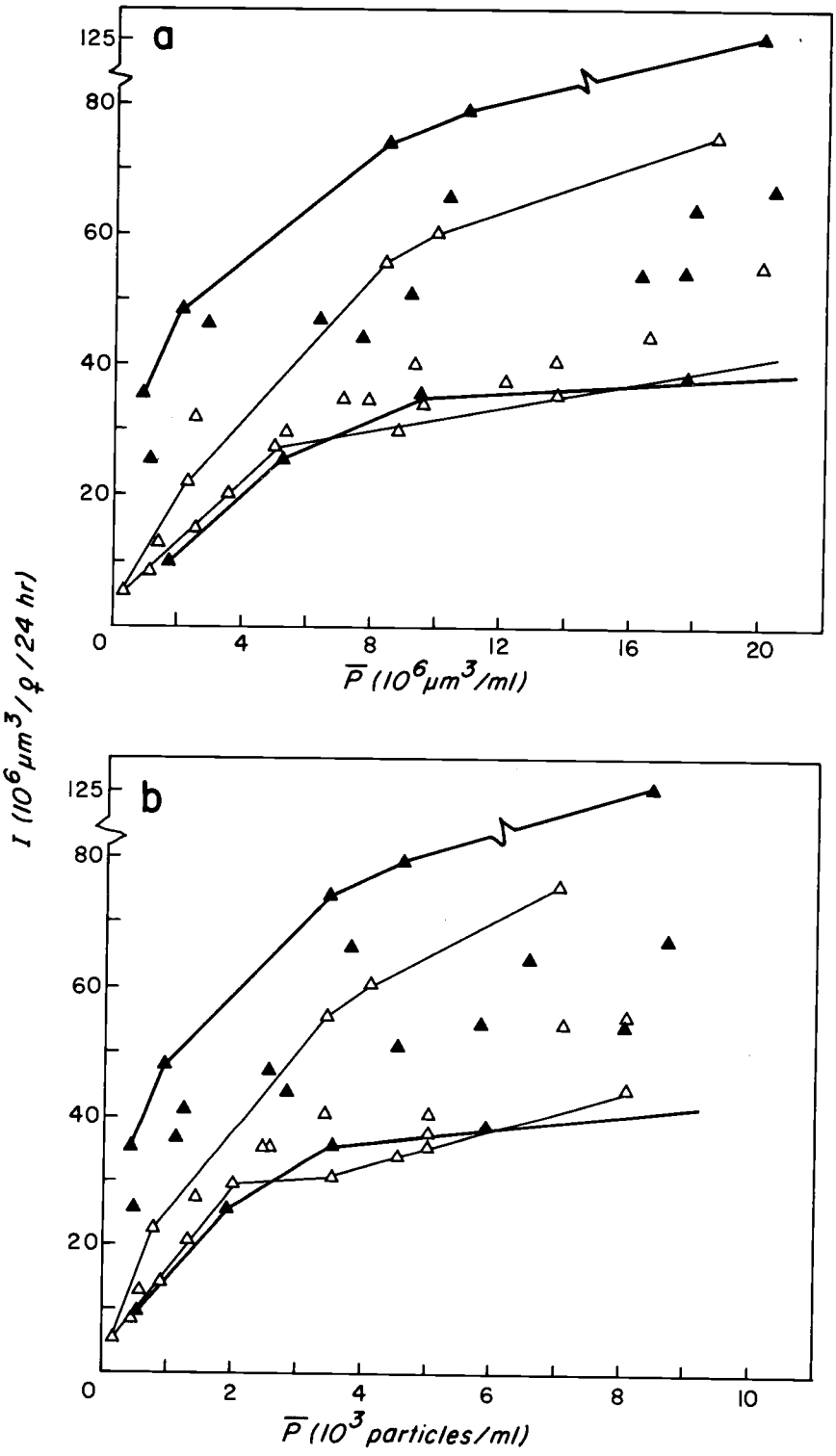

Figure 12. Comparison of T. gravida ingestion rate envelopes for up-bay, △ and down-bay ▲ A. clausi females. a, $\overline{P}$ in terms of $\mu m^3 ml^{-1}$. b, $\overline{P}$ in terms of particles ml^{-1}. Light solid line encloses ingestion rates for up-bay females, heavy solid line encloses ingestion rates for down-bay females.

analyses. These samples were taken over a biomass range of 232 × 10^6 μm^3 to 2063 × 10^6 μm^3 in the case of T. nordenskioldii, and over a range of 319 × 10^6 μm^3 to 2213 × 10^6 μm^3 in the case of T. gravida. The least squares regression equation for carbon versus volume data in T. nordenskioldii was:

$$\widehat{\mu gC} = 4.870 + 0.100\,(10^6\ \mu m^3). \qquad (5)$$

For nitrogen vs. volume data the equation was:

$$\widehat{\mu gN} = 0.903 + 0.024\,(10^6\ \mu m^3). \qquad (6)$$

The linear correlations coefficients were 0.989 and 0.984 for the carbon and nitrogen regressions, respectively. For T. gravida, the regression equations were for carbon versus volume:

$$\widehat{\mu gC} = 1.770 + 0.104\,(10^6\ \mu m^3). \qquad (7)$$

and for nitrogen versus volume:

$$\widehat{\mu gN} = 0.048 + 0.030\,(10^6\ \mu m^3). \qquad (8)$$

The correlation coefficients were 0.984 and 0.986 for the carbon and nitrogen regressions, respectively.

A number (12-24) of unfed females were collected and dried on glass fiber filters several times during the experimental period for carbon and nitrogen analyses (Figure 2b and 2c). Over the time course of the experiments, the carbon content of down-bay animals ranged from 2.384 to 4.050 $\mu gC\ animal^{-1}$, while the corresponding

nitrogen contents ranged from 0.759 and 1.154 μgN animal^{-1}. For the up-bay females, the carbon content ranged from 0.872 to 1.959 μgC animal^{-1}. The corresponding nitrogen values were 0.223 and 0.632 μgN animal^{-1}.

The phytoplankton carbon and nitrogen regression equations were used to convert the post-24-hour control and grazed suspensions to their carbon and nitrogen equivalents. These values, converted only for experiments with corresponding copepod carbon and nitrogen data, were used to calculate ingestion in terms of μgC female^{-1} 24 hours^{-1} and μgN female^{-1} 24 hours^{-1} employing equation (2). Carbon-specific and nitrogen-specific ingestion rates were computed by dividing the per-animal ingestion rates by the carbon and nitrogen contents of the animals, expressed as percent body carbon and body nitrogen eaten per day.

Carbon-specific and nitrogen-specific ingestion rates calculated for both populations of A. clausi fed on each diatom tend to be food-biomass dependent (Figure 13). The variability of the specific ingestion rates is large not only because of the variability associated with ingestion rate measurements, but also because of the high measurement variability and seasonal changes in the carbon and nitrogen content of the animals. Nevertheless, specific ingestion rates of both populations of A. clausi appear to be higher with T. gravida as food, over the range of particle volume concentrations used in my experiments.

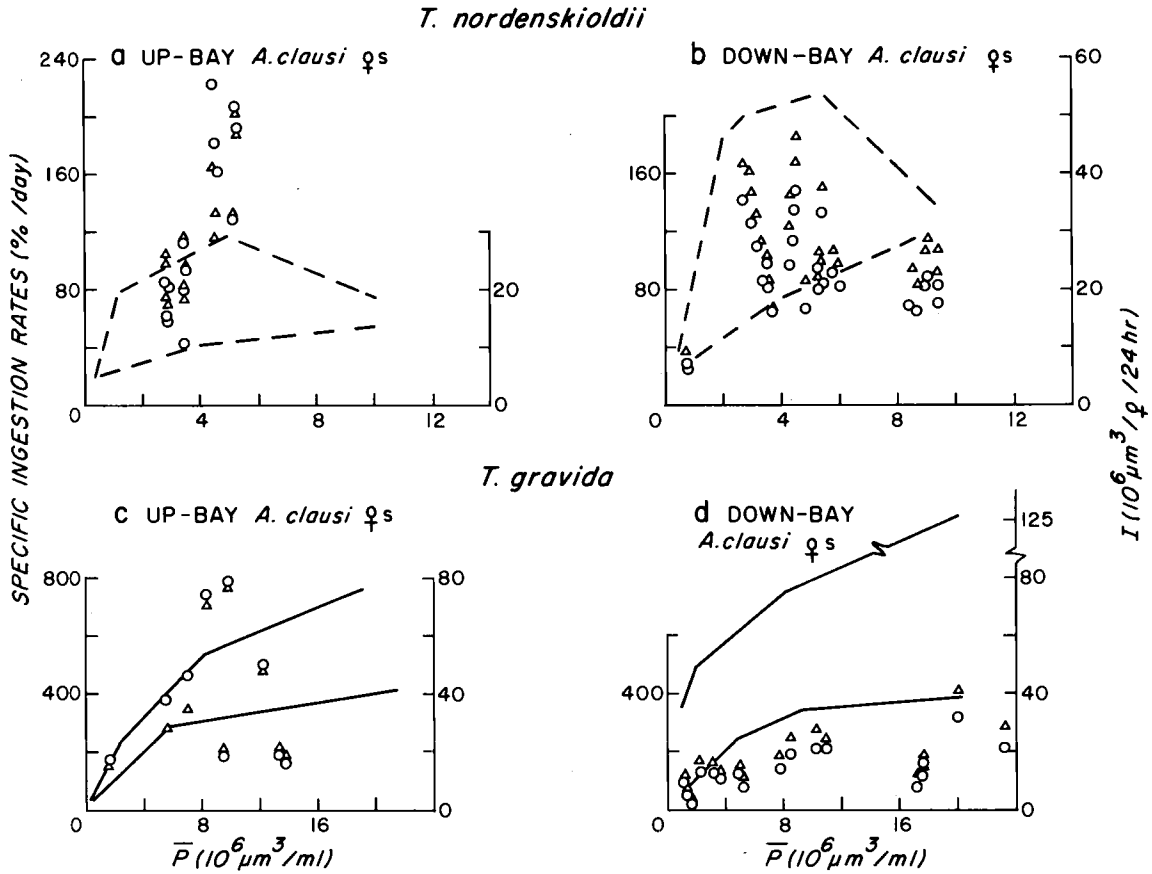

Figure 13. Specific ingestion rates: % of copepod body carbon ingested per day, Δ and % of body nitrogen ingested per day, O (left ordinate) for up-bay (a and c) and for down-bay (b and d) A. clausi females fed T. nordenskioldii and T. gravida as a function of $\overline{P}$ in terms of $\mu m^3 ml^{-1}$. The corresponding ingestion rate envelopes are plotted (right ordinate) as a function of $\overline{P}$. Note that the ordinate and abscissa scales are different for T. nordenskioldii and T. gravida.

DISCUSSION AND CONCLUSIONS

Volume size class frequency distributions

Volume size class distribution shifts in the absence of grazing activity have been reported by other investigators. Donaghay (personal communication) observed a decreasing volume size class mode and average particle volume in the marine planktonic diatom Skeletonema costatum in experiments in continuous flow systems. The S. costatum particle volume decrease was correlated with increasing nutrient supply (increasing flow rate) into the systems. Prakash et al. (1973), using batch and dialysis cultures, related changes in particle size distributions to phases of the growth cycle in S. costatum and other phytoplankton organisms. They found maximum particle size during the stationary phase and smaller particles during log-phase growth. Lundy (personal communication) observed a higher frequency of smaller S. costatum particles in rapidly aerated batch cultures. Reducing the rate of aeration increased the frequency of larger diatom particles. Curl (personal communication) has correlated larger S. costatum particle sizes with lower temperatures and nutrient depletion in laboratory experiments.

I believe that the volume size class distribution shifts observed in the absence of grazing in my diatom suspensions (Figures 4 and 5) were probably a response to the high nutrient input, via the frequent

dilution of the cultures with fresh medium. The high inputs induced a rapid growth rate; i.e., they sustained log-phase growth. The nutrient inputs were probably much higher than might be expected in the diatom's natural environment.

The volume size class distribution shifts induced by the grazing of female A. clausi (Figures 7 and 8) were confined to the larger, longer-chained Thalassiosira gravida. An explanation of this effect might be found in the way female A. clausi handle food particles. On a number of occasions, the grazing activities of both populations of A. clausi were observed microscopically. The observations were made on females not used in the experiments. The animals were placed in petri dishes containing suspensions of T. nordenskioldii or T. gravida or natural phytoplankton suspensions collected from Yaquina Bay. The use of their mouth parts by the grazers appeared to conform to Gauld's (1964) description; i.e., the first maxillipeds were used actively as a scoop net to capture particles. The spread maxillipeds were repeatedly swept forward very rapidly. This motion was often accompanied by a jerky forward movement of the animal over a distance of one to several body lengths. One to several sweeps of the maxillipeds and forward jerks were often observed to be followed by a quiescent period of short duration in which often could be seen peristaltic movement of the brownish-green food mass contained in the esophagus and foregut.

When feeding, especially in dense suspensions of phytoplankton chains, I often observed that the setae of the feeding appendages would accumulate phytoplankton particles during feeding which were remnants of diatom chains. These particulate accumulations would frequently be discarded by the animals by one to several rapid flexes of the first and second antennae and the rest of the appendages. Often these accumulated particles would be broken by the copepod's efforts to remove them. The result was to produce a number of particles smaller than those upon which the females were feeding. This phenomenon was especially prevalent when the animals were feeding upon natural assemblages of Yaquina Bay phytoplankton chiefly composed of long-chain-forming diatoms such as Chaetoceros, Skeletonema, Thalassiosira, Thalassionema, and others. Chaetoceros species were very abundant in the assemblages, and some chains were up to 500 μm long.

Such feeding activity: the scoop net activity of the first maxillipeds and the clearance of particle accumulations from the setae of the feeding appendages might explain the reduction in average particle volume in the grazed T. gravida suspensions (Figures 7e and 8e). T. gravida produced longer chains than did T. nordenskioldii, and these longer chains apparently were broken much more easily. The preferential ingestion of larger T. gravida particles, without large-particle break-up, would not explain the increased frequency of

smaller T. gravida particles in the grazed suspensions. In nature, this reduction in average food particle volume by grazing could be significant in terms of producing smaller food particles that might be more readily ingested by smaller zooplankton, including naupliar and copepodite stages of A. clausi.

Ingestion rate envelopes

The several investigators who have reported copepod ingestion rates as a function of food biomass concentration have fit by inspection either a linear function (Mullin, 1963), a curvilinear or Ivlev-type curve (Parsons et al., 1967; McAllister, 1970), or a rectilinear function (Frost, 1972) to their data. Where more than only a few data points are reported, copepod ingestion rates versus biomass concentrations show a large amount of variability, especially at relatively high food biomass concentrations. As McAllister (1970) notes, because of the variability there must be considerable uncertainty attached to any Ivlev constants (see equation 1) assigned to aggregate data.

In cases of high data variability, I believe that an envelope containing all the data points might better represent the relationship between copepod ingestion rate and food biomass concentration than any specific mathematical function. In Figure 14, I have redrawn and enveloped Frosts' (1972) data on Calanus with his rectilinear

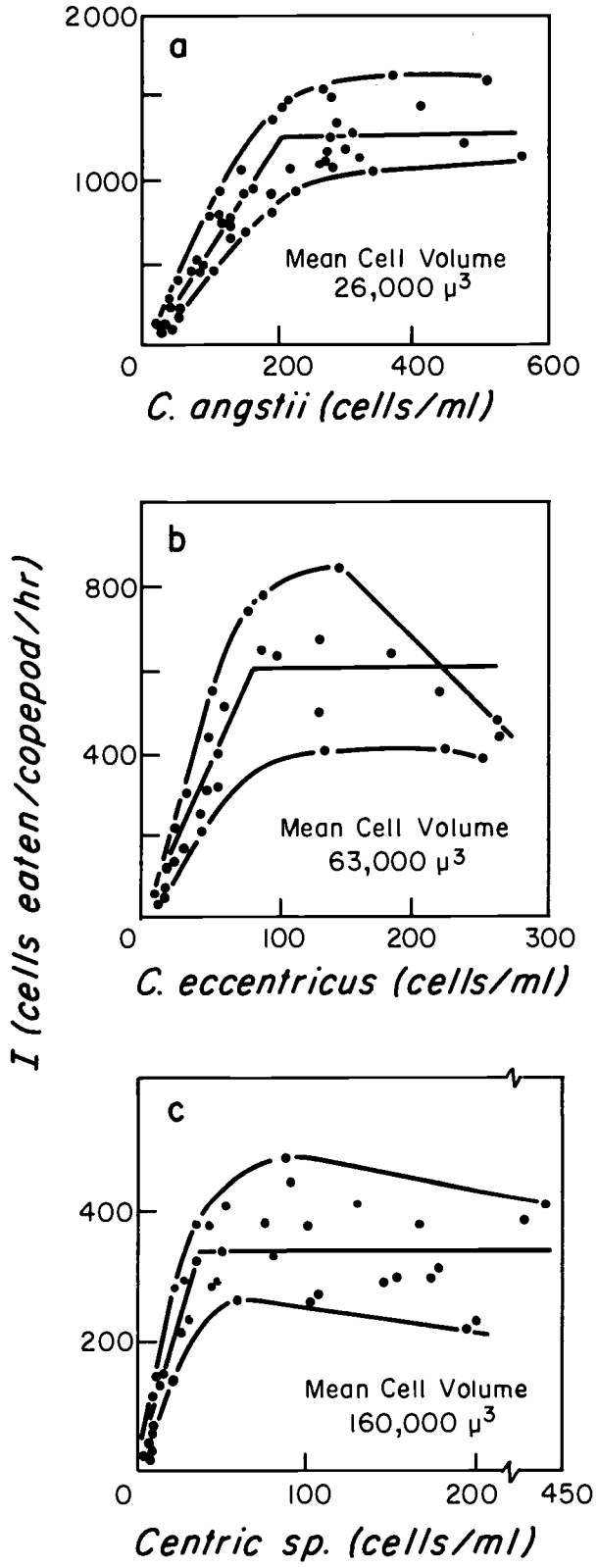

Figure 14. Ingestion rate envelopes for Calanus females grazing on three centric diatoms. Redrawn from Frost (1972). See text for explanation.

functions left in place. In two of the three plots, the envelopes appear to better represent the sense of the grazing response than do the rectilinear functions. The envelopes could be given without the data points and not introduce much misrepresentation of the ingestion rate relationship: this would not be true of two of the rectilinear functions. The often observed decline in ingestion rate beyond a certain food biomass concentration is particularly not handled by Ivlev-type equations or rectilinear representations (Figures 11 and 14).

Because the female A. clausi used in my experiments were collected over the reproductive season of late spring to late fall in 1971 and 1972, the resulting ingestion rate envelopes probably represent seasonal variability in the rates. The exclusive use of adult females in the experiments was predicated on the assumption that the females collected throughout the reproductive season would be roughly equivalent in grazing activity, without regard to the date of collection. This was probably not a valid assumption. Ingestion rate at a given food biomass concentration might be expected to vary among females that have just molted from stage V copepodites, females with maturing ovaries, and females actively laying eggs. It is noteworthy that the five data points forming the upper boundary of the T. gravida ingestion envelope estimated for the down-bay A. clausi (Figure 10) were determined using animals from one zooplankton collection taken on 21 September 1972. These five points

form a smooth curve, suggestive of an Ivlev-type relationship. This data set was the only one in which sub-groups of animals from the same collection were run over a large range of $\bar{P}$. Procedures in other experiments were such that animals from any given collection were used at only one to three food concentrations encompassing only a small range of $\bar{P}$. At the very least, my data and those of Frost (1972) indicate the incorrectness of specifying food-biomass-dependent grazing rates for a species based on only one set of rate determinations from one zooplankton collection made at one time of year.

Both the up-bay and down-bay A. clausi grazing rates on T. nordenskioldii appeared to be lower at the high food concentrations (Figures 9 and 10). Ingestion rates for Calanus feeding on Coscinodisicus eccentricus (Frost, 1972) followed a similar pattern (Figure 14b). Conover (1956) noted that a lower grazing rate was obtained in his A. clausi and A. tonsa grazing experiments at high phytoplankton concentrations, usually involving Skeletonema costatum as the food. Conover mentioned that it was not clear whether the low grazing rates he obtained during the large spring phytoplankton blooms were due to satiation or mechanical inability of the copepods to handle so much food. The cause of ingestion rate depression and its relation to food concentration, food particle size and grazing mechanisms remains an unresolved problem.

Significant differences in the ingestion rate envelopes in my experiments were obtained only when the two populations of A. clausi females were fed the smaller T. nordenskioldii (Figure 11). Envelope overlap was confined to the lower portion of the envelope estimated for the down-bay females and to the upper half of the envelope estimated for the up-bay females. In the case of T. gravida as food, the envelope overlap for both A. clausi populations was nearly complete over the whole range of $\overline{P}$ (Figure 12). These data suggest that the larger down-bay females might be able to ingest more phytoplankton over a wider range of particle volume size than the smaller up-bay animals. The smaller up-bay females might achieve higher ingestion rates in nature when larger food particles are available for ingestion. Conover (1956) noted that A. clausi grazed relatively inefficiently when the food organisms were naked flagellates and other nannoplankton.

In terms of specific ingestion rates on a carbon and nitrogen basis, both up-bay and down-bay A. clausi appeared to be able to ingest as high as 200% of their body carbon and nitrogen per day when fed T. nordenskioldii (Figure 13a and 13b). Up-bay and down-bay females appeared to attain even higher rates when fed T. gravida (Figure 13c and 13d). In the case of T. gravida where data were abailable over comparable ranges of $\overline{P}$ for both up-bay and down-bay females, the up-bay females appeared to achieve higher rates than

did the down-bay females, although data for the up-bay animals were widely scattered.

The trends of the element-specific ingestion rates tended to follow the trends of the grazing envelopes in terms of μm^3 eaten $female^{-1}$ $24\ hours^{-1}$ although data scatter was large (Figure 13). Trends were particularly noticeable for down-bay copepods, for which specific ingestion rate data were available over a larger range of particle volume concentration than for the up-bay animals.

Such high specific ingestion rates implied that the animals were "starved"; that is, their digestive tracts were empty at the beginning of the experiments. It is doubtful that such rates would be maintained for very long periods of time. However, copepods might not feed for long periods of time, and conceivably could begin each feeding period with empty digestive tracts. Also, many of the females in my experiments had maturing ovaries or were developing eggs, so that high specific ingestion rates could partly be accounted for by these physiological demands.

Figures 9 and 10 were drawn in order to determine (1) how the rates of ingestion of food biomass varied with the total food biomass concentration of each diatom (Figures 9a and 10a), and (2) how the rates of ingestion of food biomass varied with total particle concentration of each diatom (Figures 9b and 10b). I was not able to compute and compare rates of ingestion of food as particles because additional

smaller particles were continually being produced in the T. gravida suspensions as a result of grazing activity. Comparison of Figure 9a with Figure 9b showed that the envelope overlap in Figure 9b is reduced from that observed in Figure 9a at particle concentrations below about 3000 particles ml^{-1}. This reduced envelope overlap occurred because the average volume of a T. gravida particle was larger than that of a T. nordenskioldii particle. Thus, an up-bay female that ingested an average-size T. gravida particle would consume about twice as much biomass as a female that ingested an average-sized T. nordenskioldii particle. That the biomass ingestion rates of T. gravida below 3000 particles ml^{-1} in Figure 9b were, on the whole, less than twice that of T. nordenskioldii at the same particle concentration implied that the rate of ingestion of T. gravida particles was less than that for T. nordenskioldii in this particle concentration range. This condition might reflect increased "food-particle-manipulating" difficulties due to the larger average particle size of T. gravida relative to the small average particle size of T. nordenskioldii.

Food biomass ingestion is about equal for both diatoms in biomass concentrations up to about 6×10^6 μm^3 ml^{-1} (Figure 9a), which infers that the copepods must ingest greater numbers of the smaller T. nordenskioldii particles at low biomass concentrations.

The reduction in envelope overlap was not as striking as in the

case of the down-bay females, probably due to increased data variability (Figure 10a and 10b). That the food biomass ingestion of these large females was the same with both T. gravida and T. nordenskioldii as food (at least up to concentrations of 6×10^6 to 8×10^6 μm^3 ml^{-1}), is obvious from Figure 10a. This similarity infers that the rates of ingestion of particles were higher in the T. nordenskioldii suspensions than in the T. gravida suspensions. The trend toward reduced envelope overlap when biomass ingestion rates are plotted against particle concentrations (Figure 10b) tends to point up the particle-size differences between the two diatom species (in a similar fashion as Figure 9b with the up-bay copepods).

Conceivably the average particle-size differences between the two diatoms was not as pronounced in the experiments with down-bay A. clausi as in the experiments with the up-bay animals. Thus the down-bay females were able to achieve a higher ingestion rate over a greater range of particle size than the up-bay animals. The higher T. nordenskioldii ingestion rates achieved by the down-bay females (Figure 11) offset any increased T. gravida biomass ingestion rate on a particle basis (Figure 10b) that might have been due to the larger T. gravida average particle volume.

Comparable volume size class frequency distributions for grazed suspensions of T. gravida in Figures 7f and 8f peak at the same volume size class (1540 μm^3), which suggested that both up-bay

and down-bay females break up T. gravida particles in the same way. Data variability precludes further speculation on the reasons for the apparent overlap differences between Figure 9b and 10b.

The relative impacts of the two Acartia populations on the two diatom populations can be further examined by plotting final concentrations of particles in the post-24-hour controls against final particle concentrations in the grazed suspensions (Figure 15). A 45° line constructed through the origin denotes equal numbers of particles (regardless of particle size) in the control and grazed suspensions after 24 hours. Points below the line indicate that the grazed suspensions had a higher particle concentration than the corresponding post-24-hour controls at the end of the experiment; i.e., that the net effect of grazing activity and any cell division that occurred in the grazed suspension produced more particles than occurred in the post-24-hour control. Points above the 45° line indicate that there were more particles (regardless of size) in the post-24-hour control than in the grazed suspension at the end of the experiment; i.e., the net effect of grazing activity and cell division was a reduction in the total particle concentration.

Inspection of Figure 15 demonstrates that, in almost all of the experiments in which up-bay females were fed T. gravida, the particle concentrations in the grazed suspensions were higher than in the post-24-hour controls. Assuming that cell division is equal in any

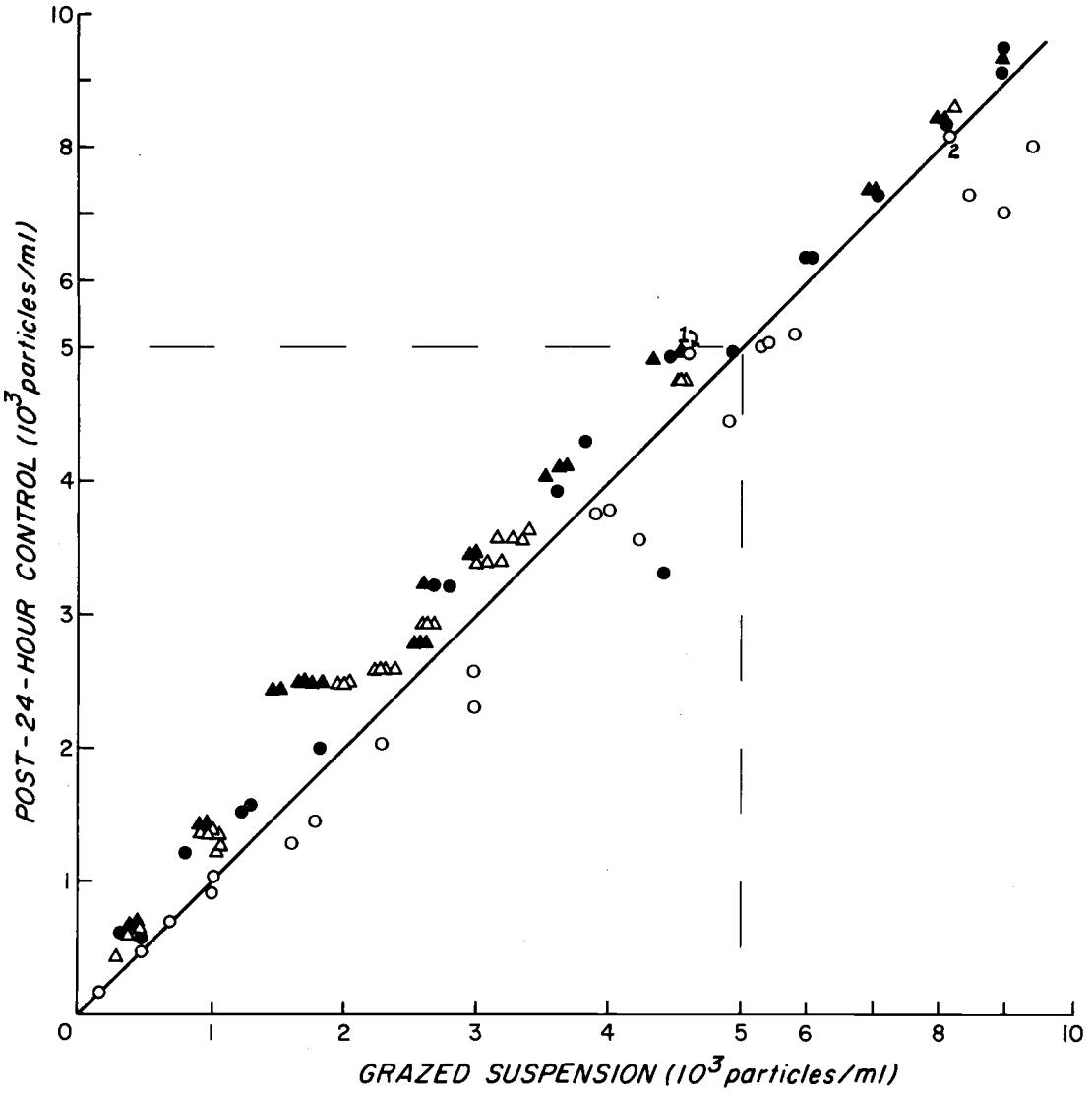

Figure 15. Particle concentration in the grazed suspensions versus particle concentration in the post-24-hour control suspensions.

	A. clausi females	
Food diatom	up-bay	down-bay
T. nordenskioldii	△	▲
T. gravida	O	●

Symbols for the data points are equivalent to the mean of three replicate particle concentration determinations ± about one standard deviation for corresponding grazed and post-24-hour control suspensions. Note that the dashed line indicates a scale change at 5000 particles ml^{-1} on both the ordinate and abscissa. For explanation of the two numbered points, see the text.

corresponding grazed and post-24-hour control suspension (which might not be exactly true), the higher particle concentrations in the grazed suspensions could only come about if grazing activity were breaking large particles into smaller ones. Inspection of Figure 7e and 7f, representative of all the experiments run with up-bay females fed T. gravida, demonstrates that the average particle volume was smaller in the grazed suspension than in the post-24-hour control. The assumption that cell division was equal in grazed and control suspensions cannot be proved, but near equality seems likely in view of the fact that all nutrients (particularly NH_4^+) were in abundant supply in all suspensions.

For particle concentrations greater than 1000 particles ml^{-1} (Figure 15), data points further below the 45° line are for those experiments run early in the T. gravida culturing period, before much of the volume size class frequency distribution shift had occurred (see Figure 6a). The two data points labelled 1 and 2 (Figure 15) were run late in the T. gravida culturing period when the diatom particles were the smallest; i. e., when the chains of cells were the shortest (see Figure 6b). This relationship suggests that the larger particles, representing longer chains, were more susceptible to being broken into chains of fewer cells by the grazing activity of the up-bay females.

For the five experiments nearest the origin in Figure 15 (below

1000 particles ml^{-1}), the data points are on the 45° line or near it. These experiments were run early or toward the middle of the T. gravida culturing period, before all of the volume size class distribution shift had occurred. At low particle concentrations, the ingestion rates of the up-bay females apparently could better match the combined effects of cell division plus the production of smaller particles through grazing activity.

Except for the data points for the up-bay copepods grazing on T. gravida and one other point, all the points in Figure 15 were located above the 45° line. The data point that falls below the 45° line is for an experiment with down-bay females grazing on T. gravida. This experiment was run very early in the T. gravida culturing, when the volume size class frequency distribution was characterized by a high frequency of larger particles. Apparently an exceedingly large amount of particle breakage took place relative to particle removal by grazing. The remainder of the points indicate that fewer particles were present in the grazed suspension at the end of the experiments than in the post-24-hour control at the same time. Removal of particles by grazing surpassed the ability of cell division and particle breakage to replace those removed. Of particular note is that all the data points for up-bay females fed T. nordenskioldii fell above the 45° line, exactly opposite to the relationship with T. gravida; thus, in the first case, up-bay animals could ingest most of the

particles without breaking them, while in the T. gravida suspensions many particles had to be broken, and the subsequent ingestion rate could not keep pace with the rate of production of additional, smaller particles.

The increased frequency of smaller particles in the T. gravida suspensions grazed by up-bay animals came at the expense of a decreased frequency of larger particles. Even so, particles in the grazed T. gravida suspensions were, on the average, larger than those in the grazed T. nordenskioldii suspensions (see Figure 7c and 7f). The up-bay females thus always had larger particles, and more of them as time progressed, in the T. gravida suspensions than in the T. nordenskioldii suspensions.

Implications of food-size modification in previous studies

If other zooplankton grazers are able to modify the volume size class frequency distribution of their particulate food, then experimental results obtained with these other grazers might require reinterpretation.

Richman and Rogers (1969) inferred that Calanus when fed the planktonic diatom Ditylum, actively hunted and selected dividing pairs of cells, but passively filtered single cells, based upon the lower frequency of paired cells in grazed suspensions when compared to controls. During a final stage of division Ditylum daughter cells are

attached only by a terminal spine on the adjacent valves of the daughter cells. In this condition, Ditylum daughter cells might be easily separated by the feeding activity of Calanus without being actually ingested by the copepods. Such mechanical separation of daughter cells in the grazed suspension would produce an over-estimate of the filtering rate of dividing pairs. The resulting increased frequency of single cells in the grazed suspension would result in an under-estimate of the rate at which the Calanus had filtered them.

Several investigators have suggested hypothetical relationships between the size of particulate food and particulate grazer ingestion in natural aquatic systems. Brooks and Dodson (1965) suggested that particle grazers compete for the smaller, more frequent particles in natural systems. The larger animals, however, might possess a greater ability for food collection and could better utilize the larger, less frequent particles. Under these circumstances the larger grazers should outcompete the smaller ones. While my experiments dealt with grazers that were taxonomically closely related and which differed in size only marginally when compared to size differences among all grazers in nature, the experimental results nevertheless, indicated relationships that differed somewhat from those hypothesized by Brooks and Dodson (1965). My data suggested that the larger down-bay female A. clausi when compared to the up-bay females, possessed only marginally greater ability to utilize the larger food particles.

This situation might have resulted from the ability of both sets of copepods to dismember large food particles. The larger females demonstrated significantly higher ingestion rates when fed the smaller diatom, however.

Schoener (1969) suggested that, if the food particle size spectrum consistently graded from very abundant small particles to very rare large particles, then the opportunity would exist for ingestion specialization by grazers. If small grazers were specialists in the ingestion of small particles, and if food was scarce (large particles being very scarce), then small grazers would be favored. But my data, and those of Conover (1956), suggested that A. clausi, a small grazer, is able to achieve higher ingestion rates on larger food particles. The larger down-bay females achieved higher ingestion rates on the smaller food diatom than did the up-bay females.

An unstated assumption in the hypotheses of Brooks and Dodson, and Schoener, is that the spectrum of food particle size remains unchanged in time. The reduction of large particles to smaller ones by grazers would be a confounding event that could invalidate this assumption. The problem of quantifying food particle size in terms of "large" and "small" in relation to grazer size and ingestion ability must remain unresolved over the time course of grazing, if food is continually being modified by the grazers.

Isaacs (1966), Sheldon and Parsons (1967a) and Parsons and

LeBrasseur (1970) have considered that the food available in aquatic systems consists of a continuous particle-size spectrum in which food biomass may be recorded as the number of particles multiplied by the volume of an individual particle in any particular size category. For example, Sheldon and Parsons (1967) found that in June in Saanich Inlet, B. C., the particle spectrum (which ranged from 268 μm^3 to 562×10^6 μm^3) consisted of peaks of biomass formed by an unidentified nannoplankton species, diatoms of the genus Chaetoceros, and eggs and adults of the copepod Pseudocalanus. Such a scheme, I believe, is a valid representation of a plankton community. However, the distribution of biomass in any such particle-size spectrum might change rapidly over short time intervals as a response to the processes discussed herein. Multiplication of particle numbers by fixed particle volumes in corresponding size categories could lead to misinterpretation of the dynamics of the community. The impact of grazing activity upon the volume size distribution of natural phytoplankton assemblages has not been investigated intensively but might be an important process in controlling the abundance and distribution of plankton in nature.

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