Effects of Crowding on Reproduction and Feeding Rates in the Cladoceran *Daphnia pulex* Leydig

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To

Ardys Richardson Cairncross

who, in another time,
would have accomplished this
with excellence

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Chapter 1

Introduction

The cladoceran Daphnia pulex was chosen for this study for the following reasons; first, its distribution is ubiquitous over the continent; second, it inhabits not only shallow ponds but also certain deep lakes; and third, a great deal of the basic biology of Daphnia is already understood. Despite this fact, there are many questions yet to be answered about the reproductive biology, feeding behavior, and especially the evolution of Daphnia. The mechanism of the switch from asexual or parthenogenic reproduction to sexual reproduction is not known, nor has any endocrine system been found in Daphnia.

Daphnia pulex Leydig 1860 emended Richard, 1896 (Richard, 1896) belongs to the Class Crustacea, Subclass Branchiopoda, Order Diplostraca, Suborder Cladocera. It features five pairs of thoracic appendages, the third and fourth of which are heavily equipped with comb-like filtering setae that traditionally have been thought to act as a seive in the filtering of algae as water is forced out past the setae (see Cannon, 1933; Jorgensen, 1966). In a recent seminar, Karen Porter has speculated, on the basis of preliminary experiments, that the structure of the filtering setae may actually represent the minimum material necessary to create an adsorptive or adhering surface or "wall" on which the food particles would collect by a surface attraction mechanism,

i.e., the water may not actually pass out between the setae. For a detailed discussion of the taxonomy of D. pulex, see Richard, 1896, and the translation in Appendix X, Scourfield, 1942, Brooks, 1953, Brooks, 1957, and Edmondson, 1959. For a description of the anatomy of Daphnia, see J.H. Lockhead in F.A. Brown, 1950.

Cladocerans are restricted almost entirely to fresh water, although a few marine species exist, e.g., Penilia avirostris Dana occurs in Kingston Harbor, Jamaica (Gore, 1980), and the genera Podon and Evadne occur occasionally in the neritic areas of the Bedford Basin, Nova Scotia (Conover and Mayzaud, 1976). Despite the presence of microfossil remains in sediment cores of post glacial lakes (Frey, 1960, 1974, Mueller, 1964), very little is known about the evolution of the group. Lake Baikal, circa 50×10^6 years old, contains 370 species of crustaceans, of which 98% are endemic, primarily amphipods (291 species), copepods, and ostracods, but no cladocerans (Brooks, 1950). Daphnia pulex has developed a "strange and manifold" pelagic existence in some North American lakes, but in Europe it is a small pond species (Woltereck, 1932). It is ubiquitous in North America south to Mexico, but it is rarely found in the very northern areas of the continent. Along the northern edge of the range of D. pulex, questionable hybrids are found with D. middendorffiana, which occurs in Arctic and Subarctic

shallow ponds, and is able to produce resting eggs, or ephippia, without the usual fertilization by males. There may be some hybridization of D. pulex with D. rosea, and some introgression into D. schödleri.

Cladocerans usually reproduce by apomictic or ameiotic parthenogenesis, one of the three basic types of parthenogenesis (Soumalainen, 1950). The other two basic types, generative and automictic parthenogenesis, are also known to occur in zooplankton. In generative or haploid parthenogenesis, found in the rotifers, meiotic reduction in the eggs produces males. In automictic or meiotic parthenogenesis in Artemia salina, the meiotically reduced cells undergo self-fusion either of the cleavage nuclei or fusion of the öocyte with the second polar body. In the type found in the cladocera, apomictic or ameiotic parthenogenesis, the egg undergoes equational division but no reduction, similar to mitosis. Some contraction of the 24 chromosomes is seen, but there is no pairing and no formation of chiasmata. This form of parthenogenesis occurs not only in the cladocera, but also in ostracods, aphids, some Turbellaria, Trematoda, nematodes, in the parthenogenic production of females in the rotifers, in the snail Campeloma, in an isopod and some insects. In aphids, the X chromosomes pair, and extrusion of one X chromosome into the polar body results in XO males with XX females, while the autosomal chromosomes undergo apomictic

parthenogenesis. In the case of the cladocerans, males have been shown cytologically to be diploid (Mortimer, cited in Banta, Wood, Brown and Ingle, 1939), and segregation of mutant characters into male Daphnia longispina (Banta, 1928) affirms the diploidy of the male.

Apomictic or ameiotic parthenogenesis can lead to long term heterozygosity, since there is no recombination, and recessives, as they arise, will accumulate. Only dominant mutations or structural rearrangements will be effective in evolution. Apomictic parthenogenesis makes polyploidy possible, since the difficulties inherent in meiotic pairing are avoided. Soumalainen (1950) suggested that parthenogenesis was selectively advantageous because it made possible polyploid races that were adapted to live in periglacial waters. Parthenogenic races of the isopod Trichnoniscus elisabethae are distributed to the north, a triploid race is found in Sweden, and a diploid race in France. It might be worthwhile to look for polyploidy in the rotifers which exhibit a size increase from south to north, e.g., the genus Trichocerca. It may be that polyploid races or species do not occur in the cladocera because of the selective advantage of the lines which could produce the resistant resting eggs, which usually require the fusion of meiotically produced eggs and sperm. In polyploidy, at least in triploidy, meiosis involves difficulties as the odd numbers of chromosomes attempt

to pair.

While an alteration of the asexual and sexual phases typifies reproduction in cladocera, the parthenogenetic phase usually predominates. The females produce young genetically identical to themselves, unless mutations have occurred. Shortly after egg laying, one diploid polar body is extruded from each parthenogenic egg (Zaffagnini and Sabelli, 1972), but this loss of genetic material does not reduce the high reproductive rates in the asexual phase in D. pulex where broods of 10-30 and more young occur. A female living through 15 - 20 instars can potentially produce 300-400 young under good conditions. There is a strong linear dependence of the number of young/brood on body length, except in aging females, where, even though the maximum size has been reached, the average number of young per brood declines (Green, 1954, 1966; Banta et al, 1939). The regression of egg number on the body length of Simocephalus was found to vary seasonally, indicating that body length was only one of the factors important in egg production (Green, 1966). It is interesting that populations established from the young of older females cannot be cultured through as many generations as those from young of the first brood, a phenomenon referred to as the "Lansing" orthoclone effect (Lansing, 1948). Addition of inositol partially reversed this curious effect of aging (Murphy and Davidoff, 1972).

The role of genetic variation in clones of Daphnia is probably substantial in growth, reproduction and feeding rates. Clones of D. longispina varied markedly in their tendency to produce "sexual eggs" and males (Wood and Banta, 1933) and differences between clones were observed in age at reproduction, number of young/brood, number of broods/female, and time between broods (Ordway, 1928). Some clones showed slower development and greater longevity (Banta et al,1939). Natural populations of D. pulex that are reproducing parthenogenetically have exhibited allozyme variation in 6 enzymes, indicating the co-existence of several clones in one habitat (Hebert and Crease, 1980). Populations of Daphnia magna in more permanent ponds showed distributions of homozygotes and heterozygotes that rarely could be fitted with Hardy-Weinberg expectations for the distribution of the genotype frequencies of their enzyme loci, heterozygotes predominated. Analysis over time showed marked shifts in frequencies and extreme instability at some loci, the cause of which was not understood (Hebert, 1978). Egg production among individuals in uncrowded clones varied by 25% (Hebert, 1974), but individuals from crowded clones varied two to tenfold in their egg production (Hebert and Ward, 1976).

The first event in sexual reproduction in cladocerans is the production of the diploid males. In 1637, Schwammerdan thought cladocerans were hermaphroditic because he saw only

one form, but in 1857, Lubbock recognized males for the first time, and suggested that sexual reproduction was environmentally controlled (Lubbock, 1857, cited in Stuart, Cooper and Miller, 1932). Anatomically, the male of D. pulex is smaller than the female, possesses a depressed head, an enlarged sensory antennule, "hairs" on the concave anterior ventral carapace edge, and an elongated and hooked 1st thoracic appendage, presumably to aid in grasping the female. The ovigerous female D. pulex may have a body length of 1.8 -2.5 mm or more. The mature male is typically around 1.4 mm in body length. The paired testes lead to a sperm duct opening near the anus in the postabdomen. In the male, the heart rate is approximately 20% faster than that of females, and longevity is correspondingly shorter (MacArthur and Baillie, 1929).

By fertilizing the eggs, the male is responsible for the production of meiotically reduced eggs. The first and second polar bodies are not released by the egg until after fertilization occurs. Fusion of the two pronuclei presumably occurs about 60 - 90 minutes after egg laying (Zaffagnini and Sabelli, 1972). The crowding cue triggering male production acts at a "critical period," one to four hours prior to egg laying (Banta and Brown, 1929-a,b). It is interesting that meiosis in the females is cued when the eggs are laid, and not, apparently, in the ovaries. The two fertilized eggs become encased in saddle-shaped protective pigmented hardened

membranes, the ephippium, which is shed at the next molt and survives desiccation and freezing. In the Arctic, D. middendorffiana produces ephippia containing diploid parthenogenetically produced eggs, but fertilization prior to maturation of the ephippia is the rule in most cladocerans.

The key of the environmental control of sexual reproduction is in the production of males, especially during the critical hours before egg laying. Whether an external cue is mediated by a hormonal or pheromonal like substance is unknown. Groups of four cells, presumably neurosecretory in function, are found clustered near the esophageal ganglion, in a frontal group, and in a group near the median, "naupliar" eye. The latter group of cells may affect ephippial production (Van den Bosch de Aguilar, 1968, 1972; Halcrow, 1969), and the quantity of "neurosecretory" material may be affected by photoperiod (Parker, 1966), but more research is required to establish clearly the functions of these cells.

The marine worm, Bonellia (Phylum Echiura) induces any of its larvae that enter it to become dwarf males, while those outside remain female. Sex is also "environmentally" controlled in the mollusc Crepidula. Here, the youngest slipper shells at the top of the stack are males, and become female as they age. In addition, they can become female if they are isolated from the group, or if females are scarce. This mode of reproduction is known as protandric

hermaphroditism.

Development in cladocerans is direct in contrast to the typical crustacean metamorphic development via a nauplius. Daphnia young are free swimming miniature adults able to feed even before release from the brood pouch. Following release of the young, there is a molt followed by laying of new eggs into the brood pouch. Growth is incremental at each molt, with the greatest increments in growth, roughly 20 -25%, occuring in the early instars. Embryological development time is strongly dependent on temperature (Lei and Clifford, 1974) and does not vary greatly from species to species (Hebert, 1978).

Size, quantity and quality of food are important determinants of growth and reproduction in Daphnia. While Daphnia are thought to feed most efficiently on cells ranging from the size of bacteria to approximately 20 micrometers diameter, they will thrive in culture on the 40 micrometer long green alga, Ankistrodesmus falcatus. The classic effects of starvation in cladocerans are as follows: greater longevity (e.g., from 28.2 days to 38.5 days in high to low food), reduced growth, longer periods between molts, or longer instars, smaller size at maturity and a reduced heart rate, e.g., 4.3 beats/sec in low food compared with 5.5 in adequate food (Banta et al, 1939; Vijveberg, 1976; Dunham, 1938). Higher food levels increase the birth rate either by

increasing the average body size, number of young per brood, or the frequency of molts.

The minimum level of food required for any growth to occur is lower than that required for eggs to be produced. Lampert and Schober (1980) have shown the minimum food required by D. pulex to grow would be above the zero growth level of 0.05 mg Scenedesmus carbon/liter. In order for any egg production to occur in first broods, a food level above 0.08 mg Scenedesmus C/liter was required, and maximal egg production occurred at .5 mg C/liter. These minimum or "threshold" food concentrations were somewhat similar for other species of Daphnia and algae, but the growth rate was reduced in Daphnia fed the diatom Nitzschia. While D. pulex is able to grow slowly on flakes of the blue green alga Aphanizomenon flos-aquae, reproduction is low and mortality high (Nancy Holm, pers. comm.). Ann Duncan (pers. comm.), working with D. thorata and D. pulicaria fed a species of Cryptomonas, found no young or eggs produced at 50 to 200 micrograms C/liter and maximum fecundity at 670 micrograms C/liter. Carbon levels in Lake Washington were much lower (20 - 30 micrograms C/liter), but turnover rates were not known.

The original plan for this thesis project was to follow seasonal changes in parthenogenic and sexual reproduction in a "permanent" deep lake and in a shallow pond population, and to make a careful comparison of the effects of food supply,

daylength, temperature and population density on the switch from asexual reproduction. The sampling program would have included quantitative sampling of the zooplankton of Square Lake, Washington Co., Mn., using a Schindler style plankton trap with duplicate samples every meter (15 -17 samples). Temperature measurements, collection of water samples for oxygen, chlorophyll and carbon analyses were to be taken at each depth. Dissolved oxygen levels are important because oxygen below 2 - 3mg/l depresses the feeding rate markedly (Kring and O'Brien, 1976, Heisey and Porter, 1977). In addition, egg production drops as O_2 falls below 2 mg/liter (Fox, Gilchrist and Phear, 1951). Oxygen levels at 2 mg/liter and below were observed in Square Lake at 13 meters and below on 4/24, 5/14 in 1977 and on 6/16 and 7/27 in 1976, with oxygen dropping only to 4.3 mg/liter at 18 meters on 5/10 in 1976. The lake was fully mixed on 11/14, 1976 with O_2 of 8.71 mg/l at the bottom. This lake showed maximum O_2 levels at 6 or 7 meters of 9 - 10mg/liter in April, May and June, indicating a deeper algal population. Secchi disc reading in May was 7.6 meters. It was common to find larger aging female D. pulex that were pink in color, indicating the synthesis of hemoglobin, in the deeper water of this lake.

Very early in the preliminary sampling it became evident that a precies quantitative description of the conditions these organisms lived in, that is, the densities, food levels,

temperatures and O_2 levels, would require frequent day-night sampling to describe the pattern of vertical migration of the Daphnia in the deep lake or the daily fluctuation of conditions in the shallow pond. The diurnal vertical migration of Daphnia in lakes assures cyclical daily changes. The migration late in the day over 3-4 meters or more (Haney and Hall, 1975) means that the Daphnia may pass through a temperature range of 12° C, through food levels over a 3-4 fold range, and through oxygen gradients ranging from 1 to 9 mg/liter, based on a study of Squaw Lake in Itasca State Park (Edwards, Helgen, Sandquist, 1975). Because the vertical migration changes seasonally, shifting sometimes to reverse migration under the ice, the pattern would have to be monitored on a regular basis. Then, predictions would be made from the more frequent midday sampling of the proportion of each day each density spent under each set of conditions of O_2, temperature and food level. The importance of each factor in the switch to sexual reproduction, including the Daphnia density, would be analyzed. The possible role of low oxygen in maintaining "successful" dominant populations of D. pulex in deeper lakes by retarding feeding and egg production would be considered.

In the shallow pond, the daily changes of conditions are more sporadic and are more controlled by environmental changes and less so by the behavior of the Daphnia. Frequent if not constant monitoring of temperature and dissolved oxygen

would be necessary because of wide daily fluctuations. Quantitative sampling of Daphnia in the shallow pond is more of a challenge because the large volume Schindler style plankton trap might not be useful.

Preliminary sampling made it abundantly clear that the proposed study required a team of workers, and it was decided that it would be more fruitful to work with cultured Daphnia. In the laboratory the factors of density, food level, temperature and photoperiod could be controlled and varied as desired. Does crowding of Daphnia stimulate the shift to sexual reproduction as suggested by Banta's work, and that of Hill (1965), and if so, is the effect separable from that of food competition in a crowded population, or could the Daphnia be emitting some factor or chemical signal into the water that triggers gamogenesis?

Banta (1925) and Banta and Brown (1929a) demonstrated that crowding of the cladoceran Moina macrocopa was important in stimulating male production, whereas the amount of food was minor, because parthenogenic females in crowded cultures continued to produce large broods. The time of the crowding was critical: males were produced when females were crowded four hours before egg-laying; isolation of females in this period resulted in female production (Banta and Brown, 1929b). When food levels as well as cladoceran density were varied, increased crowding resulted in more males at

the lower food levels, but crowding was the more important factor. Food concentration had no effect on male production in Daphnia pulex under short day lengths (9L:15D, 12L:12D) and low temperatures, i.e., 16° (Stimpfl, 1971).

Crowded female Moina macrocopa isolated at 1/7.5 ml produced as many males as those maintained at 10/75 ml (Banta and Brown, 1929). In contrast, male production by crowded D. pulex at a density of 8/24 ml was substantially higher than at 1/3 ml (final density of 333/liter), suggesting that some kind of interaction was involved (Stimpfl, 1971). When Stimpfl connected the Daphnia in a density of 8/24 ml to a 300 ml volume of medium with a net barrier, male production was reduced by half, indicating a possible dilution of some chemical.

Complex water-mediated interactions have been observed in other zooplankton, e.g., the protective morphogenic change to spines induced prior to egg cleavage by a non-dialyzable factor in the rotifer Brachionus by its predator, Asplanchna (Gilbert, 1966). The allelopathic inhibition of feeding rates in copepods where even the water that had contained the predatory and herbivorous copepod Epischura caused a reduction in the filtering rate of Diaptomus, the stronger filter feeder of the two species (Folt and Goldman, 1981). The allelopath causing such "interference competition" between the copepods was not dialyzable.

The second phase of sexual reproduction, the production of the ephippium, may be more sensitive to food depletion than is male production (Stuart, Tallman and Cooper, 1931; Stuart, Cooper and Miller, 1932; Banta et al, 1939). It is commonly known that ephippia occur in cultures that are allowed to "run down" by not changing the medium or adding food, although Wood and Banta (1933) found it necessary to add a little food. Under the important conditions of short days and low temperature, D. pulex produced more ephippia in low food (Stimpfl, 1971). On the other hand, other experiments indicate that short days combined with high density of D. pulex were required for ephippial production. No ephippia were produced at 60 Daphnia per liter, and 59% at 250/liter (Stross and Hill, 1968).

The difficulty in separating food level effects from Daphnia density effects can be seen in data showing a strong linear relation between sexual induction and density (Hill, 1965). The cultures were fed 125 x 10^6 cells every 48 hours. If the feeding rates did not change with the density of the Daphnia, and assuming an hourly ingestion rate of 40,000 cells $D^{-1}h^{-1}$, the Daphnia at 60/liter would consume almost the total amount added, whereas those at 600/liter would have exhausted their food supply, and would have been able to consume 10 times the amount given. Whether this reduced consumption per crowded Daphnia was enough to cause ephippial

production is not known, but because food was not in excess at the end of the 48 hours, it cannot be ruled out that the stimulation of ephippial production came from food limitation and perhaps not from the density. Most of the work on reproduction in cladocerans has been carried out with pulse feedings, commonly at 24 or 48 hour intervals, with the result that there is a continuously changing level of food, so that firm statements relating egg production to constant food levels are rare. Even recently in the experiments of Goulden and Horning (1980), two food levels are created by feeding Daphnia at 10,000 cells/ml every two days vs. 100,000 cells/ml every four days. Despite the fact that a 24 hour pulse feeding may mimic the natural regime of a vertically migrating organism just as closely as a continuous flow system, there is a need for data on reproduction at constant food levels. Studies on the minimum food level necessary for any egg production as well as for maximum egg production are being carried out (see Lampert and Schober, 1980; A. Duncan, pers. comm.; Hrbácková and Hrbácek, 1978).

To understand the interrelation, if any, between crowding and feeding on reproduction, and to look for water born signals of crowding, four kinds of experiments were designed. In the first set, crowded and uncrowded Daphnia were separated by isolating the crowders within net-covered cages that were immersed in the medium that contained uncrowded Daphnia.

Uncrowded Daphnia were given an excess of algal food and their reproductive rates and sex of their young were recorded. Any factors emitted from the crowded Daphnia would have contact with the uncrowded Daphnia, and the uncrowded animals would not compete with the crowded ones for food. In the second experiment, reproductive rates and male production were followed in low and high density populations fed so that each was feeding at the maximum rate in order to eliminate the factor of food limitation, and to allow any metabolic signal to accumulate. The medium was not changed, and feedings were made by direct resuspension of algae from a Millipore filter. In the third set of experiments, a careful study of feeding rates over a wide range of cell concentrations was carried out at various densities of Daphnia to determine whether crowding was affecting the feeding rate. The mathematical functions used to describe feeding were reviewed, and a feeding function for D. pulex that includes the observed density dependence in the feeding rates was derived. The fourth experiment was designed to test for allelopathic effects on feeding rates in uncrowded test Daphnia placed in water previously conditioned by crowders with appropriate controls. An analysis was made for suspected induction of mortality in the algae by Daphnia conditioned water.

Chapter 2

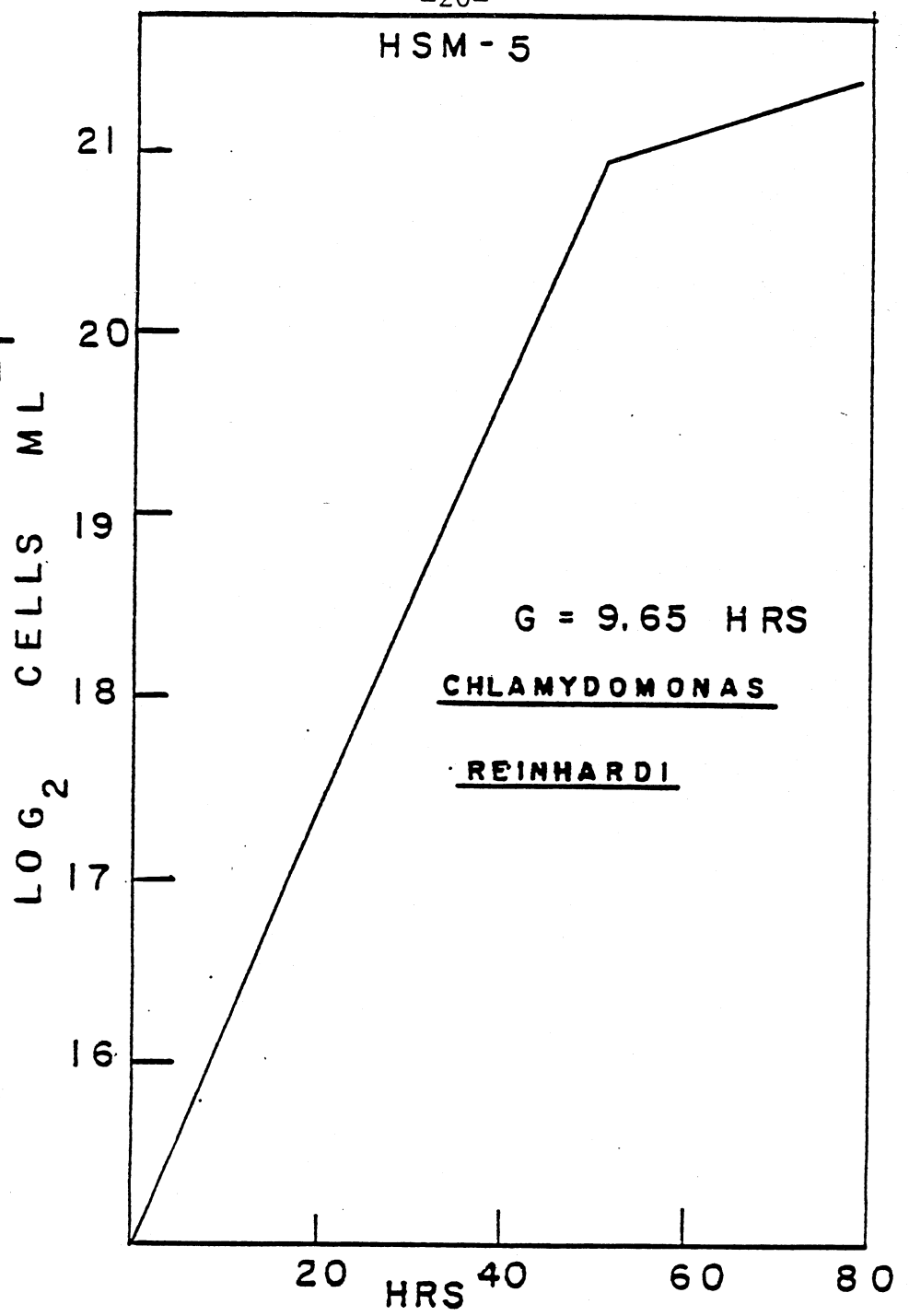
Methods

The methods used in culturing the algae and *Daphnia* are outlined in this section, and methodology used in specific experiments will be described in the chapters on each experiment.

The green alga, *Chlamydomonas reinhardi*, UTEX strain 90 from the Starr collection of algae now housed at the University of Texas in Austin was used in most of the experiments. Originally, the green alga, *Ankistrodesmus falcatus* v. *acicularis*, UTEX 101 cultured in Gorham's ASM-1 medium (Appendix I) was used to feed *Daphnia* successfully, but *C. reinhardi* was the species of choice because it was easier to count, and more uniform in size. *Chlamydomonas reinhardi* does not grow well in ASM-1 medium, but thrives in Sueoka's HSM medium as well as in the modified medium HSM-5 developed here for direct feeding of *Daphnia*. Apparently, *C. reinhardi* lacks the enzyme nitrate reductase needed to use the NO_3^- ion contained in ASM-1. Sueoka's HSM is a better medium because it contains NH_4^+ as the nitrogen source. Figure 1 shows the log linear growth of *C. reinhardi* up to 2×10^6 cells/ml with an average generation time of 9.65 hours in the HSM-5 medium. The development of this medium will be discussed later in this chapter. Some effects on *Daphnia* fed *C. reinhardi* cultured in ASM-1 under high light are described in Appendix II.

Chlamydomonas reinhardi is biflagellated and phototactic

Figure 1. Growth curve of Chlamydomonas reinhardi in HSM-5 medium.


HSM - 5
21
20
19
18
17
16
LOG2 CELLS ML-1
G = 9.65 HRS
CHLAMYDOMONAS
REINHARDI
20
40
60
80
HRS

so cells are able to layer at the surface of a non-turbulent lighted culture. Loss of the flagellae, as during cell division, will cause the cells to sink to the bottom of the culture. The algal cells are not entirely uniform in size because occasionally the daughter cells remain attached together for a short period, and the cells enlarge before dividing. Measurements of Chlamydomonas cells that had been dark adapted overnight showed cell volumes ranging from 54 to 268 μm^3. Culturing C. reinhardi in a light: dark cycle can lead to synchrony of cell division, which could cause systematic changes in cell size, as well as in cellular protein content which is highest at the end of the light cycle in synchronized cultures (Surzycki, 1971). The net increase in cell protein per cell occurs only during the light cycle, and it shows a linear increase from 1 to 12 hours in the light (Surzycki, 1971). In developing artificial particulate matter to fulfill the nutritional requirements of Artemia, Moina, and Daphnia, Conklin and Provasoli (1977) found that Daphnia could not grow well on the high starch/protein ratio of 5:1 used for Artemia particles. Instead, Daphnia and Moina required starch/protein ratios between 1.5:1.0 and 0.5:1.0. Because it was not feasible to routinely harvest the algae at the end of their light cycle to obtain high protein cells for the Daphnia, it was decided to use continuous light to prevent synchronous reproduction and thus to have a more uniform protein content

whenever the algae were used.

In C. reinhardi, sexual reproduction leading to thick walled resting cells (zygotes) results from nitrogen deficient culture and removal of the N source is routinely used by algal geneticists to induce gametic production. The addition of nitrogen to deficient cell cultures inhibits fusion of the gametic cells as they rapidly dedifferentiate (Sager and Granick, 1953). Cells shifted from highly active photosynthesis to the dark produce large visible starch grains that produce cells with a lumpy surface, particularly when grown in an acetate medium or under N deficiency. Chromosomal DNA replication occurs several hours in advance of the next mitotic division, which explains why cultures placed in the dark for several hours may double in number. Therefore it was necessary to dark adapt the algae for 12 hours before they were used in feeding experiments.

To prevent synchronous cell division, C. reinhardi was cultured in constant light from a bank of two 38W cool white fluorescent bulbs positioned to provide 540-600 ft. ca. light at the level of the liquid cultures. The culture temperature was maintained at $21^{\circ} \pm 1^{\circ}$ C. Excellent growth and resuspension of the cells was maintained by bubbling the sterile one liter flasks with either 5% CO_2/air mixture or with air passed through a column of charcoal/glass wool. In either case, the gases passed through a high pressure Millipore

filter holder containing sterile cotton, a prefilter pad and a 0.2 micrometer Metricel filter placed just before the air entered the sterile algal cultures.

The culture medium used for C. reinhardi was my modification of Sueoka's HSM (Sueoka, 1960). To this simple but highly concentrated medium (Appendix III) was added Huttner's trace metal mix at 1 ml/liter prepared by the method of Surzycki (1971) and the vitamins biotin, B_{12}, and thiamine HCl. The media for algae were made up with glass distilled water previously metal-distilled and passed through a Barnstead Combination Cartridge Model D 8922.

Lack of adequate refrigerated centrifuge capacity for mass cultures made it desireable to feed stock Daphnia the entire algal culture, including medium. This practice is avoided by most culturists because of possible toxic effects in the algal medium. Early tests with the media showed that Daphnia dies rapidly, within 2-3 hours, in algae in HSM diluted 1:100 with glass distilled water. In 1:4 diluted algal stock in HSM, death occurred in 30 -40 minutes, in 10 minutes in undiluted straight stock. Sterile unused HSM was toxic at 1:4 and 1:2 dilutions, and only at 1:10 dilution of HSM with aged tap water was it nontoxic overnight. The media appeared to be somewhat less toxic to newborn Daphnia than to older Daphnia. Daphnia cannot tolerate the salinity of the ocean. The median survival toxicity of Daphnia pulex in NaCl

is 50mM, the salinity of the ocean is roughly 599 mM, the total molarity of HSM is 23.1 mM.

Substitution of Na^+ salts for most of the K^+ salts in the modified HSM was important for successful culture of Daphnia. In devising culture media for Daphnia, Taub and Dollar (1964) found that the K^+ ion was toxic, particularly when Na^+ was low, with KNO_3^- > KH_2PO_4 > KCl in toxicity. Tests of survival of D. pulex in mixed salt solutions showed that the ratio of Na^+ : K^+ was crucial. Five mM KCl was very toxic by itself, but was nontoxic when combined with 5 mM NaCl. KCl at 10 mM was completely toxic, fewer than 1% of the Daphnia surviving for 24 hours, and .05 mM nontoxic over 24 hours. The authors preferred a Na^+:K^+ratio of 21.

The removal of K_2HPO_4 from HSM for algae culture and the substitution of a combination of NaH_2PO_4, Na_2HPO_4 and KH_2PO_4 in reduced quantity lowered the K^+ level to .207 g/liter, or 5.29 mM which when diluted 1:10 in Daphnia feedings would be close to the nontoxic level. Growth of C. reinhardi in two modified HSM media was excellent, but the HSM-Na medium containing a molar ratio of 105.6 Na:K resulted in vacuolations in roughly one fourth the cells, whereas HSM-5 with a Na:K ratio of 5 gave uniformly healthy cells, and it was the medium of choice. The growth of the algae in HSM-5 was logarithmic to 2.02×10^6 cells/ml, that is, the plot of $\log_2$ of cells/ml against hours was linear (see Figure 1). Algae

were used at concentrations usually around 1 x 10^6 cells/ml up to about 1.5 x 10^6/ml at the time of harvest. Generation time was excellent, between 9.5 and 9.8 hours. These cultures were used successfully to feed the Daphnia stocks directly, with the precaution that the total algal medium added between changes of tank water would not be more concentrated than a 1:10 dilution of the algal medium, although on occasion, this became somewhat more concentrated by the end of the week when the tank water was replaced.

Algae in the feeding experiments were harvested by Millipore filtration using Gelman 0.2 micrometer Metricel filters GA-8 (63025) prerinsed with 100 ml glass distilled water. Following filtration, algae were rinsed immediately with a few ml of medium, and resuspended.

Cell counts were made with a Sedgewick Rafter slide and a calibrated ocular reticle disc so that lengthwise and crosswise counts of the slide could be made. Cells preserved in I/KI solution (Appendix IV), or killed with 1:20 formalin were allowed to settle at least 10 minutes after filling of the slide before counting. Usually at least two lengthwise or crosswise passes of the slide would be counted, 3 - 5 for more dilute concentrations, or a total of approximately 300 cells at low concentrations, and over 1000 at high cell levels. Counts using the Sedgewick Rafter slide and the ocular disc were far quicker than those with the hemocytometer,

which had to be cleaned and refilled several times at low cell concentrations. Cell counts based on triplicate counts generally had an error range of around 5%, but this was variable.

Because of the error range in the cell count method, the use of fluorimetry as well as spectrophotometry was explored. The Beckman model DB-G was not sensitive enough at low cell concentrations when the standard three cc cuvette was used, e.g. the larger alga Ankistrodesmus falcatus at 33,000 cells/ml had an $O.D._{665}$ of .024, the lowest cell concentration tested for a regression of

$$y = 3.22 \times 10^{-7}x + .0145$$

where y is $O.D._{665}$, and x is cells/ml, and the correlation r is .984. The use of in vivo fluorescence of the algae was also explored, and correlations were high between cell counts and F readings (see Appendix IX for the methodology). Cell levels as low as 1000/ml could be detected, but there was no improvement in the error range. Because it was more convenient to preserve the cells during the experiments for later analysis, the cell count method was used.

Successful mass culture of Daphnia pulex requires a sense of humor, patience, hard work and luck, as well as the proper conditions of food and water. Daphnia is good condition swim actively, are clean and clear in color, have masses

of blue-green eggs in the brood pouch and a green upper intestine. A cascade of lipid droplets is seen in the mid-lateral region. Unhealthy Daphnia produce no eggs or gray-black eggs, are lethargic, milky in color, with perhaps a clear upper intestine and even loss of antennal parts. The latter was never observed. An infection filling the carapace in the head region with large white masses of cells was found in the Square Lake population of D. pulex, but given adequate food in the laboratory, the Daphnia grew out of it. Epibiotic infections build until the molt occurs. An interesting example of this occurred in adult overwintering females brought to the lab from Square Lake, placed in Medium D-64 and fed. The carapace of these Daphnia became thickly covered with masses of filamentous algae up to 1 mm long. The massive load of epiphytes was shed at the molt, and did not reappear. In another instance, the fungus Amoebidium parasiticum became established and attached in fairly large numbers to the swimming antennae and filtering appendages, but this burden did not affect feeding rates in repeated experiments. There were occassional periods when masses of debris including small motile rod-shaped bacteria and fine fungal cells adhered to the filtering appendages, whether this was harmful or even a defined infection is unknown.

The stocks of Daphnia pulex used in these experiments were collected from Square Lake, Washington County, Minnesota

where the species is dominant the year around. Stocks, replenished twice a year, were maintained in two 10-gallon aquaria aerated with filtered air, and filled with aged tap water dechlorinated with sodium thiosulfate and aerated for a few days. Aged tap water was stored in five and 10 gallon nalgene carboys. Photoperiod was 14:10, temperature 16-17°. As previously described, the stocks were fed at least five times a week using the algal cultures directly to a 1:10 dilution of the algal medium. The tank water was changed weekly by siphoning through a net-covered plastic cylinder. The tank bottom was cleaned with a siphoning tube. Successful stocks cleared the algae from the water overnight, whereas the few times the stocks were in trouble, the tank would still be turbid the next day.

The water source used in preparing the culture media for these experiments was extremely important. Water distilled in a metal still killed all test Daphnia within 24 - 48 hours whereas aged tap water was nontoxic. Glass distilled water was satisfactory when made up as a defined medium, and when stored in glass, not plastic, containers. All glassware was cleaned with Haemosol detergent, rinsed 6-8 times in hot tap and three times in distilled water.

The basic medium used for Daphnia culture was D-64 (see Appendix V) used by F. Hosseinie (pers. comm.) to "make Daphnia sing." After autoclaving, precipitated particles

appeared, and the medium had to be Millipore-filtered in order to make it particle-free for feeding experiments. Ultimately, autoclaving was omitted, and sterile-filtering was the rule. The simplified medium that worked well for feeding experiments was D64 made up half strength ("½D64") without the vitamins and growth factors in order to reduce any bacterial growth.

On rare occasions, Daphnia would be caught on the surface film, creating a serious problem in a feeding rate study. The addition of the surfactant bovine serum albumin fraction V to 0.1 mg% often solved this problem, but it was not used routinely because of the potential for promoting bacterial growth. The Daphnia were measured with a calibrated ocular micrometer at 40x magnification in the usual manner from the anterior edge of the head to the inflection at the base of the posterior spine. Incubations were carried out in an incubator with temperature controlled within a degree ($\pm$ 0.5°) or less in the cultures when being handled in an experiment, $\pm$ 0.1° when undisturbed. At 15° the brood time of Daphnia was around 4.7 days brood to brood; the cycle took about 2.4 to 2.5 days at 20°. The embryonic stages are described in Appendix VI. The maximum length recorded for these D. pulex was about 3.5 mm, and maximum brood size was 41 young. These large females grew from the overwintering Daphnia brought from Square Lake to lab culture. More typically, the size of

stock Daphnia used in experiments ranged from 2.2 - 2.5 mm. Brooks (1957) gives a size range of 1.5 - 2.5 mm, and Richard (1896) gives 1.8 - 2.5 mm for D. pulex. The only variant of D. pulex described to have a size over 3 mm is D. pulex hatata, but other features, such as the slight ventral sinuosity, of this variant do not fit the characteristics of Square Lake D. pulex.

Chapter 3

Experiments With Caged *Daphnia*

The following experiments were designed to determine if crowding of *Daphnia pulex* causes the parthenogenic females to increase the production of males, possibly by the release of some factor into the water. Starvation may be an important factor in gamogenesis in *Daphnia*, and because heavily crowded populations may be susceptible to intense competition for food, it is important that the *Daphnia* receiving any "signal" from the crowders be well fed. The crowded *Daphnia* can be separated from the well fed, uncrowded test *Daphnia* by a porous net barrier that would allow any chemical signal to pass out to the surrounding culture water occupied by the test *Daphnia*.

The crowders were placed in 243 micrometer Nitex nylon net-covered open-topped plexiglas cages glued with silicone, or in dialysis tubing depending on the desired pore size. The internal diameter of the cages was 1.9 x 3.4 cm, and the volume about 10 cc submerged. An outline for a simple pilot test is Table 1. The experiment was run for 15 days Young were collected and sex was determined from day four on, for the intervals of day 4-6, 6-8, 8-10, and 10-15. All test *Daphnia* were carrying several eggs (3-6) or embryos. The temperature was approximately 22°, the photoperiod was 14L:10D and light intensity at culture level was 441 ft. ca. Culture water was replaced every other day with a mixture of aquarium

Table 1. Pilot experiment with caged Daphnia and uncrowded test Daphnia.

Jar	# Cages	# Daphnia per cage	Test Daphnia per jar	Size of test D.	Jar Vol.,ml
A	2	50	10	2.21 mm	200
B	2	0	10	2.19	200

aquarium green algae, mostly Chlorella, and glass distilled water. For most experiments in this study, axenic Chlamydomonas reinhardi was used. At each change of medium, young were counted and removed to vials to mature 3-4 days before being sexed, and replacements added to the cages to maintain the density of 50/cage. These Daphnia tended to float on the surface, so the surfactant bovine serum albumin fraction V (BSA) made up in Sueoka's HSM was added at a final concentration of 1 mg%.

Important differences between treatments did occur. Average daily young production was substantially higher by a factor of 2.4x in control females in jar B through day 10 compared to those in jar A with crowders, whereas mortality was high in jar A females, 30% through day 10, 90% through day 15. There was no mortality in control jar B over 15 days. After six days, the females in control jar B had increased in size, had eggs of good color, while those in A had four of nine with empty brood sacs and some with epiphytes and fungi

attached to the carapace. The results are presented in Table 2. "Female days," or $f_i d_i$, are computed as the number of females at the end of the time interval times the length of the interval in days, or if mortalities occurred during the interval as

$$\text{female days} = f_i d_i + M_i \left(\frac{d_i}{2}\right) \quad \text{where } M =$$

the number dying in the interval. The average daily reproduction, then, is

$$\text{average } yf^{-1}d^{-1} = \frac{y_i}{f_i d_i}$$

Average daily reproduction per female is clearly depressed (Table 2) when females are in the presence of crowders (Jar A), 41.5% and 55.8%, through day 10 and 15 respectively, compared with that of the control. Another important result is the effect of the cages with crowders upon young born to test females outside the cages and reared separately in fresh medium and food. Table 3 shows the size and reproductive status of newborns, 24 hours old or less, collected on day four and reared in fresh medium until day eight. Although the difference in the average size is not significant using the Student's t test, most females from B appeared to be one instar beyond those from A. Indeed, 78% of the female

Table 2. Pilot experiment with cages: rates of young production and mortality of test females.

Jar A (with crowders)

Interval days	Females at end interval	d_i	$f_i d_i$	y_i	$yf^{-1}d^{-1}$	Average $yf^{-1}d^{-1}$
4-6	9	2	19	49	2.579	
6-8	9	2	18	10	.556	
8-10	9	2	16	33	2.06	1.736
10-15	7	5	20	68	3.4	2.192
Total				160		

Jar B (control)

4-6	10	2	20	47	2.35	
6-8	10	2	20	115	5.75	
8-10	10	2	20	89	4.45	4.183
10-15	10	5	50	181	3.63	3.927

Table 3. Size and egg production of young born in Jars A and B and reared 4 days in fresh medium

Jar	$\bar{X}$, mm day 8	S.D.	# f.f. with eggs	Total eggs present
A	1.566	.211	1/31	6
B	1.856	.153	18/23	105

juveniles born in control jar B and reared separately had reached the first reproductive instar, while only one female born in A, the largest, was carrying eggs. Birth sizes were not recorded. It appears that the young born into A medium were retarded by one molt, when compared with young born at the same time into B medium. Apparently, the effect of the A medium on the newborns is not reversible even though they are transferred within 24 hours of birth to fresh medium and food.

To complete the pilot experiment, crowded Daphnia were placed in dialysis tubing in culture jars with uncrowded test Daphnia to test for effects on the reproduction of the uncrowded test Daphnia. The control had dialysis tubing containing algae and uncrowded Daphnia. The jars are described in Table 4. The same algae mix and stock Daphnia

Table 4. Dialysis pilot experiment.

Jar	#Daphnia/tube	Test Daphnia/tube	Vol,ml
C	100	10	200
D	0	10	200

were used as described previously. Dialysis tubing, 1¼" wide with a filled length of 2½", was presoaked in glass distilled water for one hour before the experiment. A serious problem with surface tension required the use of BSA at 1mg%. Algal

growth was visible within the control tubes, and the tubing was slippery to the touch. The results are presented in Table 5.

Table 5. Results of pilot dialysis tubing experiment, young production and mortality.

Interval days	f_i	d_i	$f_i d_i$	y_i	$yf^{-1}d^{-1}$	Average $yf^{-1}d^{-1}$
Jar C (with crowders)						
3-5	10	2	20	21	1.05	
5-7	10	2	20	25	1.25	
7-12	9	5	47.5	223	4.96	3.074
			87.5	269		
Jar D (control)						
3-5	10	2	20	9	.45	
5-7	10	2	20	32	1.6	
7-12	4	5	35	103	2.943	1.92
			75	144		

Little or no inhibition of reproduction of uncrowded test Daphnia occurs when crowders are encased in dialysis tubing (Jar C). Average reproduction in C, 3.07 $yf^{-1}d^{-1}$, is comparable to that in control jar B, or 3.93 $yf^{-1}d^{-1}$. Reproduction in control jar D appears to have been inhibited compared with the previous control, and some mortality occurred.

Dialysis tubing acts as a barrier to the inhibitory effects of crowders on uncrowded Daphnia that were observed when net covered cages were used. Since dialysis tubing excludes the passage of spherical molecules of less than around 10,000 m.w., the inhibition in the cage experiments is at least not caused by a small molecule such as NH_3. Dialysis tubing, of course, would also hold back fecal matter and bacteria. No significant male production was observed, and one male was produced among 189 young.

The experiment with the net covered cages was run again with replicate jars, using new cages with 53 micrometer netting and twice the number of crowders in the cages. Incubation temperatures were 20 - 20.5^{o} , and the photoperiod was reduced to 12:12 to be within the range shown by Stross(1969) to induce ephippial production in Daphnia. The test Daphnia selected were of similar body lengths. All were carrying eggs or young, 4-19 each. Densities and lengths of the females are presented in Table 6.

Every two days the uncrowded females and the cages were transferred to fresh algae diluted appropriately with aged aquarium water. Each day, mortalities of the adults were noted and young were collected, counted and removed to rearing jars for later sex determination. Mixed stock algae were at 3.12×10^6 cells/ml at the beginning of the experiment, and ranged from $1.3 - 1.5 \times 10^6$ cells/ml from day three on.

These cell counts were made with a hemocytometer slide with a total count of 250 - 300 cells per sample. Food levels in the jars were initially 1.56 x 10^6 and ranged from .6 - .9 x 10^6 cells/ml at subsequent transfers.

An unexpectedly large depletion of cell counts occurred in the medium outside the cages in the experimental jars B and D with crowders, even though the medium was changed bi-daily. In these jars, the original concentrations of algae

Table 6. Densities in second cage experiment, 53 micrometer netting.

Jar	Cages/jar	Daphnia per jar	Daphnia per jar	Test D. BL, mm	S.D.	Vol ml
A	2	0	10	2.479	.214	200
B	2	0	10	2.509	.214	200
C	2	100	10	2.492	.208	200
D	2	100	10	2.479	.192	200

of 1.56 x 10^6 cells/ml were reduced in two days to .2 and .4 x 10^6 cells/ml, whereas the levels in the control jars A and C dropped only to 1.07 and 1.26 x 10^6 cells/ml. Even within one day, jars with crowders in cages showed a reduction in algae from .7 to .19 or .22 x 10^6 cells/ml. Because it was intended that the uncrowded females be fed at similar levels in all jars, jars B and D (with crowders) were given

supplementary feedings on the days between transfers to fresh food, using known amounts of algae filtered onto 0.2 micrometer Millipore filters, and rinsed immediately into the culture jar. Such supplementary feedings did not quite equalize the food regimes between the control and experimental Daphnia but did prevent algal levels in B and D from dropping below .11 x 10^6 cells/ml.

The decline in algae in the medium surrounding the cages in B and D may have been caused by turbulence that would have carried algae into the cages where they would be rapidly consumed by the crowded Daphnia, although the jars were usually undisturbed. Although no turbulence was visible, there could have been agitation of the medium created by the crowded Daphnia or by vibrational disturbance. Another possibility is that the crowded Daphnia promote mortality of the algae, not by consumption, but by making the water toxic to the algae. In a later experiment, a toxicity in crowded Daphnia medium is established as a cause of algal mortality. This will be discussed in Chapter 9.

Of the total of 2305 young collected from the test females during the 11 day period, none developed into males. Daily reproductive rates and mortalities are presented in Table 7. Reproductive rates are calculated as described previously, with the omission of reproduction during the first three days under the assumption that this reproduction was

not affected by the experimental conditions. The overall birthrates for the period are given in Table 8. The average brood per female is derived by multiplying the brood cycle time of 2.625 days times the average daily rate. A Student's

Table 7. Daily rates of reproduction in test females in control jars A and C and jars with crowders, B and D.

Interval days	Control $yf^{-1}d^{-1}$		Exptl $yf^{-1}d^{-1}$	
	A	C	B	D
4-5	5.503	5.200	5.700	5.333
5-6	11.370	10.333	5.500	8.589
6-7	2.000	17.167	5.400	1.125
7-8	7.714	3.500	6.300	5.375
8-9	19.429	10.667	7.333	14.857
9-10	1.715	4.400	7.125	2.833
10-11	6.429	4.000	8.000	10.167
11-12	14.170	12.000	6.750	3.273

Table 8. Overall reproductive rates of test females from control jars A and C, experimental jars B and D.

	Controls $yf^{-1}d^{-1}$		Exptl $yf^{-1}d^{-1}$	
	A	C	B	D
	8.541	8.408	6.513	6.444
Brood Aver/ female	22.42	22.07	17.10	16.92

test on the overall reproductive rates presented in Table 8 for the two replicate jars and two treatments results in a treatment difference (+ crowders) significant at the .005 level ($p < .005$), i.e., the crowded Daphnia did depress reproduction significantly in uncrowded test Daphnia.

The differences in average brood size seen here, approximately 22 young/Daphnia in controls compared with about 17 young/Daphnia in the experimentals may be the result of the unexpected reduction in algae levels mentioned previously. A rough approximation using a volume conversion to equivalent levels of Chlamydomonas reinhardi gives an initial food level of 8300 cells/ml and 3800 cells/ml at the lowest level. If these conversions to Chlamydomonas equivalents are correct, then the reproduction here is higher than that observed by Richman (1958) for D. pulex fed C. reinhardi. At 25,000 cells/ml there were only 6-8 young/Daphnia, and broods averaging 22 young/female were not reached until the level of Chlamydomonas was 100,000 cells/ml (Richman, 1958). Even if the conversions are not meaningful, Richman's work did demonstrate that the reproductive rate is quite sensitive to the concentrations of food.

The reduction of brood size in uncrowded test Daphnia was accompanied by reduced growth in the females in the presence of crowders compared to the growth in the control females. While the average size of the test females was the

same at the beginning of the experiment (see Table 6), the control females grew on the average 0.61 mm, while the experimentals grew 0.44 mm in length, the Student's t test on the difference in growth in A and C compared with that in B and D is significant at $p < .002$. Because of the strong dependence of brood size on body length, the difference in size could account for the reduced reproduction observed in females in jars B and D. An increase of 0.2 mm body length in Simocephalus vetulus can cause brood size to increase from roughly 11 to 17/female (Green, 1966). If food was not a limiting factor in these experiments. that is, if the animals were able to feed at maximum rates in excess food, then the reduction of reproduction in the presence of crowders may have been caused by whatever reduced growth. The experiment in Chapter 9 shows that crowded Daphnia do depress the feeding rate in uncrowded Daphnia. This could possibly reduce the growth rate, which in turn would reduce reproduction because of smaller body length.

In the previous two experiments, no male production was stimulated by caged crowders in test females. In the follow- experiment, the medium was not changed in order to allow any factor or factors to accumulate in the medium, and algae were added directly to the cultures from Millipore filters. The densities and sizes are presented in Table 9. Crowders were placed either in the main medium (A) with test Daphnia singly

in five cages, or in cages (B) with five test Daphnia in the main medium. The culture media were preconditioned with the crowders for two days before addition of test females on day three. Daphnia were fed and young removed daily on days four, five and six. Young were removed to rearing vials on days seven, eight and nine for sex determination.

Table 9. Test for stimulation of male production by crowded Daphnia with no change of culture medium.

Jar	# cages	#D/cage	#Daphnia outside cages	Test D. size, mm	Vol ml
A	5	1	400	2.35	200
B	2	200	5	2.31	200

The test females were chosen carefully for both size and reproductive stage of their embryos: late stage III or early stage IV embryos showing cephalic development and no primitive eyespots (see Appendix VI) were chosen to shorten the time until the first brood was released since it was the next brood that was of interest. The young collected four days after the females were added came from broods laid after the hatching of the embryos carried at the initiation of the experiment. The culture medium consisted of the stock algae diluted with aged tap water to around $.8 \times 10^6$ cells/ml. Daily counts of algae revealed that the Daphnia in cages removed

(or were toxic to) substantial numbers of cells from the medium outside the cages: up to 151 x 10^6 cells were removed each day. Crowders in A essentially removed all cells each day, or about 198 x 10^6 cells.

The results were that no males were produced in the 322 total young collected from the affected broods of the 10 uncrowded test females. By day 9, uncrowded females in B were healthy and averaging over 20 young/female, while four of the five females in A were dead.

From this set of preliminary experiments it was concluded that crowded Daphnia do not release into the medium a chemical that stimulates uncrowded females that are well fed to produce males, the first event in the switch to sexual reproduction. Whether crowding reduces parthenogenic reproduction by reducing growth or by inhibiting the ingestion rate will be dealt with in a subsequent chapter of feeding rates.

Chapter 4

Experiment on Reproduction in Crowded and Uncrowded *Daphnia*

The previous experiments with crowded *Daphnia* in cages showed that production of males was not induced in uncrowded test *Daphnia* separated from crowded *Daphnia* by a net-covered barrier. There was some repression of reproduction and growth in these daphnids, and an apparent inhibition of the development rate in their young. The effect was prevented by placing the crowders in dialysis tubing.

In the following experiment, reproduction in low (10/liter) and in high (270/liter) density populations of *D. pulex* is compared under conditions of semi-continuous feeding. Culture medium was not changed, in order to allow the accumulation of any factor produced by crowded *Daphnia* that might regulate reproduction directly, or as a consequence of regulation of the growth and/or feeding rates. Densities and feeding regimen are presented in Table 1. Axenic *C. reinhardi* strain 90 cultured in HSM-5 medium and *Daphnia* in D64 medium were used. The temperature was 15° and the light cycle 10L:14D. Stock *Daphnia* were reared under similar conditions except aged tap water was used instead of D64 made with glass distilled water.

The young were removed, counted and saved for sex determination each day from the high density, and bidaily from the low density cultures. Mortalities were recorded and the

culture volumes were adjusted as deaths occurred to compensate for the mortality and thereby maintain the density.

Table 1. Experiment on reproduction in crowded and uncrowded Daphnia, densities and feeding rates.

Jar #	Original Vol, ml	# of Daphnia	Density #/liter	# feedings per day	final cells/ml
10-1	1500	15	10	1X	30-40,000
10-2	"	"	"	"	"
10-3	"	"	"	"	"
10-4	"	"	"	"	"
10-5	"	"	"	"	"
10-6	"	"	"	"	"
270-1	200	54	270	2X	125,000
270-2	"	"	"	"	"

At 15°, the brood cycle lasted 4.7 days compared with a cycle of 2.625 days at 20.4°. The brood production of 14 isolated females is presented in Table 2. The average brood size of these females was 11.5 young/female, and the average daily rate of reproduction was 2.447 $yf^{-1}d^{-1}$. In the experiment, the young collected during the first five days were considered to belong in a "preaffected" period, those collected after five days were from eggs "affected" by the conditions of the experiment.

The preaffected period lasted from 10/31 - 11/5, the affected period from 11/6 - 11/7. Because of high mortality in

Table 2. Broods of 14 isolated females at 15°. M = molt.

Day 31	1	2	3	4	5	6	7	8	9	10	11	12	Tot# Broods	Tot y	Aver.# /brood
27	0	0	0	13	0	0	0	0	14	0	0	0	3	54	18
18	3	0	0	0	2	0	0	0	12	0	0	13	4	48	12
0	14	0	0	0	8	0	0	0	11	0	0	0	3	33	11
3	0	0	0	2	0	0	0	2	1	0	0	2	4	10	2.5
0	0	0	9	0	0	0	10	0	0	0	0	0	2	19	9.5
13	0	0	0	M	0	0	0	28	0	0	0	23	3	64	21.3
0	0	0	3	0	0	0	4	0	0	0	8	0	3	15	5
M	0	0	0	M	0	0	6	0	0	0	0	1	2	7	3.5
0	23	0	0	0	13	0	0	0	15	0	0	0	3	51	17
0	0	0	M	0	0	0	19	0	0	0	11	0	2	30	15
M	0	0	0	M	0	0	0	17	0	0	0	15	2	32	16
0	0	0	1	0	0	0	15	0	0	0	16	0	3	32	10.7
0	13	0	0	0	6	0	0	0	24	0	0	0	3	43	14.3
0	0	0	3	0	0	0	7	0	0	0	M	0	2	10	5

Average young/brood, 11.5. Average young $f^{-1}d^{-1}$, 2.48.

the uncrowded cultures, these were terminated on 11/13. The crowded cultures were shifted on 11/13 to low food and fresh medium. The medium had not been changed during the previous two weeks. At the shift, the crowded cultures were given 1/6th the amount of food, i.e., 125,000 cells/ml every three days instead of twice a day. No food, but fresh medium was given on 11/13, and subsequent feedings occurred on 11/14, 17, 20, 23, 26 and 29 with changes in media. The periods were as follows:

Period of young collection	Feeding regime
10/31 -11/5 preaffected per.	Full food, Table 1
11/6 - 11/13 affected per.	Full food through 11/12 Low food 11/13 - 12/1

During the first two weeks the original D64 medium was not changed, but a slight amount of turnover occurred because of losses during collection of young. These losses were minimized by the use of net covered pipettes.

Semi-continuous feeding of the cultures was used, the low density cultures were fed every 24 hours, and the high density cultures every 12 hours. Feeding levels were chosen so that the cell levels would not drop below 20-30,000 cells/ml to assure high rates of feeding and reproduction. Typically, low density cultures were fed daily to a total cell concentration of 40,000 cells/ml, and high density cultures

of 40,000 cells/ml, and high density cultures were fed every 12 hours to a total of 125,000 cells/ml. Maintaining a minimum food level without changing the medium presents the dilemma wherein the uncrowded Daphnia, simply by their low total daily food consumption, reduce the cell concentration much less than does the high density population; the result is that daily replacement of fresh cells would be much less in the low density cultures. In actuality, maintaining the cell level at around 40,000 cells/ml in the low density jars required 26-85% cell replacement, and complete replacement for the last four days. The crowded cultures typically required 80-90% replacement twice a day.

Prior to most feedings, cell counts for each culture were made on five ml aliquots fixed with I/KI solution and counted in a Sedgewick Rafter slide after a minimum settling time of 10 minutes. Typically, at least 300 cells were counted. The algae, axenic C. reinhardi, were harvested during the log linear growth phase, or at concentrations under 2 x 10^6 cells/ml, and rinsed on prerinsed 0.2 micrometer Millipore filters, and resuspended immediately into the Daphnia cultures.

The actual food levels before feeding and the replacement amounts are presented in Tables 3 and 4.

The average daily production of young is calculated using the number of females surviving at the time of collection

or f_i, averaged with the number present at the preceding collection interval as equal to f_a. The method for the calculation of the "period" reproduction rates for the affected and preaffected periods is outlined in the manner below.

The total production of young is given in Table 5. In all, over 6000 young were collected from the high density cultures, and over 2500 young from the low density Daphnia. Daily production and period reproduction rates are presented in Tables 6a, 6b, 7 and 8, with a summary of the average rates of reproduction for the preaffected and affected periods presented in Table 9. Period reproduction rates are calculated as shown:

$$fd_p \text{ or female days over the period} = \frac{(f_a h_i)}{24}$$

$$yf^{-1}d^{-1} \text{ for the period} = \frac{\text{total } y_p}{fd_p}$$

where y_p = total young in period

f_i = number of females at the end of interval$_i$

f_a = average number of females over the interval, or

$$\frac{f_i + f_{i-1}}{2}$$

p = period, e.g., affected or preaffected

Table 3. Food levels in low density cultures 10-1 through 10-6. Average cell levels are given.

Date	Aver. cells/ml before feeding	Aver. replacement, cells/ml	Final level, approx.	% cell replacement
10/31	21296	18704	40000	.47
11/1	28265	11735	"	.29
11/2	39402	0	"	0
11/3	29512	10488	"	.26
11/4	28729	13271	42000	.32
11/5	17227	27773	45000	.62
11/6	23230	21771	"	.48
11/7	6668	38332	"	.85
11/8- 11/12	replaced total	45000	"	1.00

Table 4. Food levels in high density cultures, averages of 270-1 and 2, fed twice daily.

Date	Aver. cells/ml before feeding	Aver. replacement, cells/ml	Final level approx.	% cells replaced
10/31	11075	90500	100000	.91
11/1	33582	66418	"	.66
11/1	84493	15507	"	.16
11/2	21359	78641	"	.79
11/2	18647	106353	125000	.85
11/3	28968	96032	"	.77
11/3	15972	109028	"	.87
11/4	18044	106957	"	.86
11/4	8664	116336	"	.93
11/5	14503	110498	"	.88
11/5	14691	110309	"	.88
11/6	33300	91700	"	.73
11/6	8702	116298	"	.93
11/7	19475	105525	"	.84
11/7	19702	105299	"	.84
11/8	18157	106843	"	.85
11/8	no count	fed total	"	-
11/9	11791	113210	"	.91
11/10	19212	105789	"	.85
11/10	no count	fed total	"	-
11/11	8777	116223	"	.93
11/11	no count	fed total	"	-
11/12	4455	120555	"	.96

Table 5. Total young production in low (10/liter) and high (270/liter) cultures by dates.

Date	270-1	270-2	10-1	10-2	10-3	10-4	10-5	10-6
10/31	88	85						
11/1	142	181	135	57	113	103	130	108
11/2	108	214						
11/3	36	80	3	42	42	44	32	12
11/5	87	72	81	28	60	67	50	66
11/6	96	194						
11/7	115	78						
11/8	189	169	81	66	72	98	88	120
11/9	112	155						
11/10	164	273	27	38	107	36	25	16
11/11	190	116						
11/12	132	197	94	36	20	55	70	107
11/13	160	224	30	17	40	44	45	29
		Total:	451	284	454	447	440	458
11/14	95	150			Total overall:		2534	
11/17	403	412						
11/19	221	286						
11/20	149	61						
11/21	104	95						
11/24	109	87						
11/26	71	25						
11/29	36	27						
12/1	75	10						
Tot:	2882	3191	270-1,2		Total overall:		6073	

Table 6a. Reproductive rates for low density cultures 10-1, 2 and 3 during preaffected and affected periods.

Cult.	Date	f_i	h_i	fd_p	y_p	$Yf^{-1}d^{-1}$ period
10-1	11/1	15	44			
	11/3	15	45.25			
	11/5	15	50	87.03	219	2.516 (preaff.)
	11/8	15	76.75			
	11/10	15	55.5			
	11/12	13	47.75			
	11/13	12	22.75	122.36	232	1.896 (aff.)
10-2	11/1	14	44.5			
	11/3	12	44.5			
	11/5	12	51.0	76.49	127	1.660 (preaff.)
	11/8	12	76.5			
	11/10	10	42.5			
	11/12	8	48.5			
	11/13	8	24.25	84	157	1.869 (aff.)
10-3	11/1	15	45			
	11/3	15	44.25			
	11/5	15	51.25	87.81	215	2.448 (preaff.)
	11/8	14	76.5			
	11/10	14	42			
	11/12	12	49			
	11/13	12	22	108.3	239	2.208 (aff.)

Table 6b. Reproductive rates for low density cultures 10-4, 5 and 6 during preaffected and affected periods.

Cult.	Date	f_i	h_i	fd_p	y_p	$Yf^{-1}d^{-1}$ period
10-4	11/1	15	45.5			
	11/3	15	44			
	11/5	15	50.75	87.66	214	2.441 (preaff.)
	11/8	14	77.25			
	11/10	14	41.5			
	11/12	12	49.5			
	11/13	8	21.75	106.8	233	2.182 (aff.)
10-5	11/1	15	46			
	11/3	15	43.75			
	11/5	15	50.75	87.81	212	2.414 (preaff.)
	11/8	15	77.5			
	11/10	14	41			
	11/12	10	50			
	11/13	7	22.25	106.1	228	2.149 (aff.)
10-6	11/1	15	46.5			
	11/3	15	43.75			
	11/5	15	51.25	88.44	186	2.103 (preaff.)
	11/8	15	77.0			
	11/10	13	40.5			
	11/12	11	51.0			
	11/13	7	21.5	105.3	272	2.583 (aff.)

Table 7. Reproductive rates for culture 270-1, during preaffected and affected periods.

Date	f_i	h_i	fd_p	y_p	$Yf^{-1}d^{-1}$ period
10/31	54				
11/1	53	25.5			
11/2	53	28.25			
11/3	53	17.0			
11/4					
11/5	50	48.0	259.8	373	1.436 (preaff.)
11/6	49	25.5			
11/7	49	22			
11/8	49	24			
11/9	49	26			
11/10	48	23.5			
11/11	47	23.5			
11/12	46	24.5			
11/13	46	30.0	398.6	1168	2.930 (affected)
11/14	43	30.5			
11/17	42	72	582.6	1666	2.860
11/19	42	53			
11/20	42	28			
11/21	42	32.35			
11/24	39	74.75	324.5	583	1.797 (shift)

Table 8. Reproductive rates for culture 270-2 during preaffected and affected periods.

Date	f_i	h_i	fd_p	y_p	$Yf^{-1}{}_{period}d^{-1}$
10/31	54				
11/1	54	25.5			
11/2	54	28.75			
11/3	54	16.0			
11/4					
11/5	54	48	266.1	475	1.785 (preaff.)
11/6	54	25.5			
11/7	53	22			
11/8	53	24			
11/9	53	26			
11/10	52	23.5			
11/11	52	23.5			
11/12	51	25			
11/13	51	29.5	435.5	1406	3.228 (aff.)
11/14	50	19			
11/17	47	72	620.97	1986	3.169
11/19	45	52.5			
11/20	45	28.25			
11/21	44	32.35			
11/24	43	75	349.5	529	1.514 (shift)

Table 9. Summary and averages of period rates of reproduction in low and high density Daphnia cultures.

Culture	preaff. $Yf^{-1}d^{-1}$	affected $Yf^{-1}d^{-1}$	shift $Yf^{-1}d^{-1}$
10-1	2.516	1.896	
10-2	1.660	1.869	
10-3	2.448	2.208	
10-4	2.441	2.182	
10-5	2.414	2.149	
10-6	2.103	2.583	
Aver. 10/1	2.277	2.151	(brood size 10.76)
270-1	1.436	2.930	1.797
270-2	1.785	3.228	1.514
Aver. 270/1	1.612	3.086 (brood 15.43)	1.650

and h_i = hours in interval i

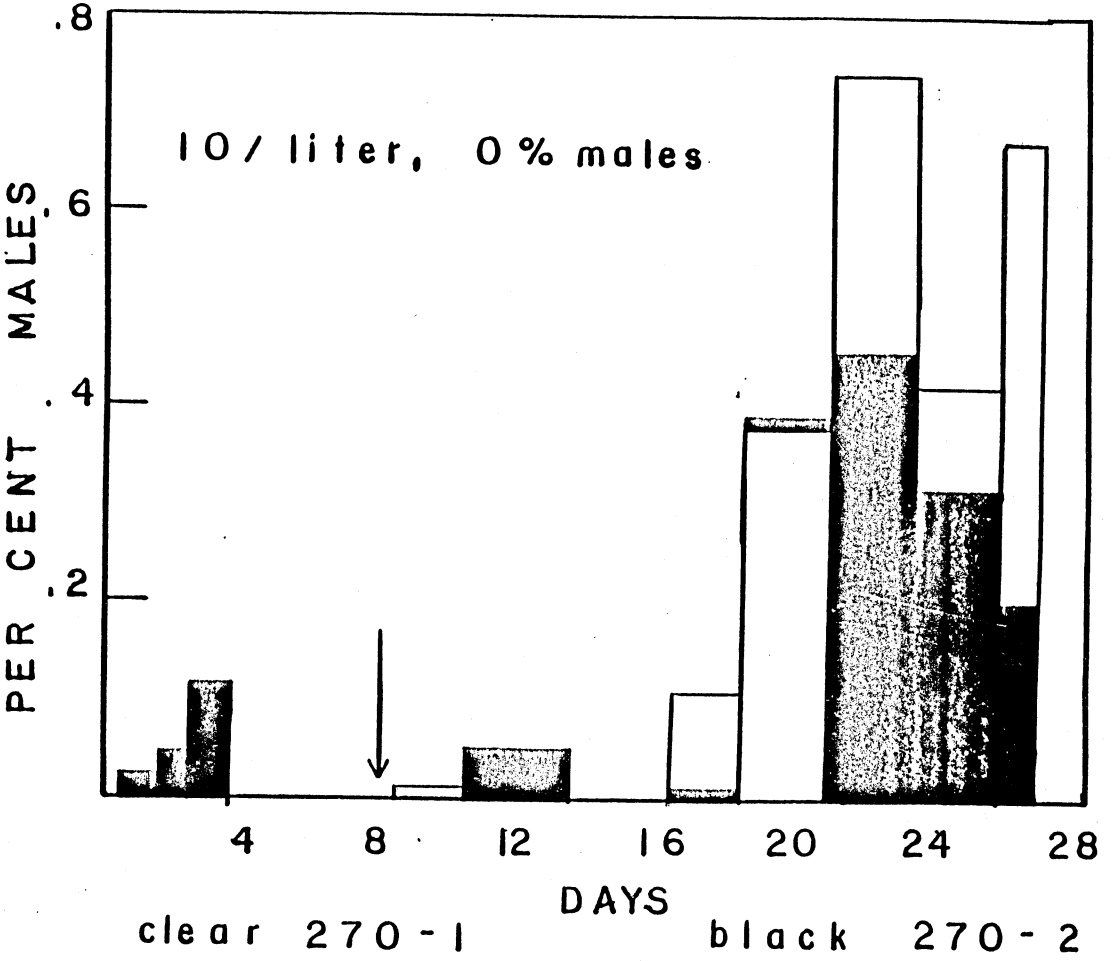
The low density cultures showed very little difference in average reproductive rate between the preaffected and the affected period, a small net decrease of 0.126 $yf^{-1}d^{-1}$. The overall rate during the affected period, 2.151 $yf^{-1}d^{-1}$, means the average brood size was 10.76, and is comparable to the rate of 50 isolated females averaging 2.62 $yf^{-1}d^{-1}$ and to that of the 14 isolated females studied for the brood cycle of 2.48 $yf^{-1}d^{-1}$. The high density Daphnia, on the other hand, showed an increase in reproduction during the affected period:the averaged rate increased from 1.474 $yf^{-1}d^{-1}$ to 3.086 $yf^{-1}d^{-1}$, or to a brood size of 15.43 young/female. When the shift to low food and fresh medium was made, the reproductive rate declined in the crowded Daphnia to an average of 1.65 $yf^{-1}d^{-1}$, or an average brood size of 8.25. These rates are based on young collected from 11/19 on, or six days following the shift in feeding on 11/13. The reduction is primarily the result of the decrease in food supply after the shift to low food. There was no evidence that the age of the females was affecting the brood size, at least the average rate through 11/17, 3.019 $yf^{-1}d^{-1}$, is not markedly different from that observed up to 11/13 of 3.086 $yf^{-1}d^{-1}$.

The sex of the young born during the entire experiment is listed in Table 10 as % males and shown in Figure 2. The uncrowded Daphnia produced no males during this period. A

Table 10. Sex of young born in low and high density Daphnia cultures during preaffected, affected and shift periods, given as % males produced.

Date	270-1	270-2	10-1	10-2	10-3	10-4	10-5	10-6
10/31	0	0						
11/1	0	.159	.027	0	.119	0	.091	0
11/2	.157	.139						
11/3	0	0	0	0	0	0	0	0
11/5	0	0	0	0	0	0	0	0
11/6	0	.016						
11/7	0	.044						
11/8	0	.113	0	0	0	0	0	0
11/9	0	0						
11/10	0	0	0	0	0	0	0	0
11/11	0	0						
11/12	0	0	0	0	0	0	0	0
11/13	0	0	0	0	0	0	0	0
11/14	.01	0						
11/17	0	.05						
11/19	0	0						
11/20	0	0						
11/21	.108	.011						
11/22	.381	.384						
11/26	.737	.455						
11/29	.419	.316						
12/1	.676	.200						

Figure 2. Male production during the affected period in 270-1 (clear) and 270-2 (black) cultures. Affected period lasts until day 8 (arrow) during which time there were no males produced in the 10/liter cultures. Males produced from day 12 on are the result of the shift to low food.


.8
.6
.4
.2
PER CENT MALES
10 / liter, 0 % males
4
8
12
16
20
24
28
DAYS
clear 270-1
black 270-2

maximum percentage of 11.3% occurred in the crowded cultures. Although crowding is stimulating some shift to male production, it does not appear to be a powerful factor, e.g., in culture 270-1 only 1% males were collected on one date, in 270-2 1.6%, 4.4% and 11.3% occurred on three consecutive dates. However, it is important to note that this stimulation to produce males by crowding is having its effect when the Daphnia are well fed, measured by their high average reproductive rates during the affected period.

Shifting the crowded culture Daphnia to low food and fresh medium on 11/13 caused a large positive response in male production in the young from 11/21 through 12/1, with a maximum of 73.7% males in culture 270-1 on 11/26. Unfortunately the mortality in the low density cultures forced a termination of these cultures on 11/13, so a comparison in response with that in high density cultures cannot be made. Low food meant that the crowded cultures were given 1/6th the previous amount, or 125,000 cells/ml every three days instead of twice a day.

Mortality of reproducing females differed considerably in the low vs. high density cultures. Daily mortality rates for the crowded Daphnia remained low even after the shift to low food rations which would be expected to affect the animals after 11/13. Even 18 days after the shift to low food, crowded Daphnia had a mortality rate of only 2.2% per day.

It appears that the crowding protects them against the effects of aging, and that low food supply is not a large factor in Daphnia mortality. As was outlined in the Introduction, Daphnia have reduced heart rates and live significantly longer in low food or starvation conditions, an obvious advantage in the natural environment. Pratt (1943) also found lower mortality in higher density (100/liter) Daphnia. Survivorship and mortality rates are presented in Tables 11 and 12 and plotted in Figure 3.

In the uncrowded cultures, the mortality increased rapidly after 11/10 in all the cultures, and reached an average mortality rate of over 18% by 11/13. During the affected period, the uncrowded Daphnia had an overall mortality of 37.9% whereas in the crowded Daphnia the loss was only 6.7%. By 12/1, 32 days after starting the experiment with mature females, 63.9% of the crowded females were still alive, and after 44 days 13 of the females were still surviving. In contrast, only 60% of the uncrowded females survived for 14 days.

The difference in the percentage replacement of fresh algal cells in the low and the high density cultures (Tables 3 and 4) may be an important factor in the observed differences in mortality. The lower density cultures received a lower percentage of replacement of fresh cells and showed a higher mortality. Ryther (1954) observed increasing mortality in Daphnia magna beginning after 10 days' culture in

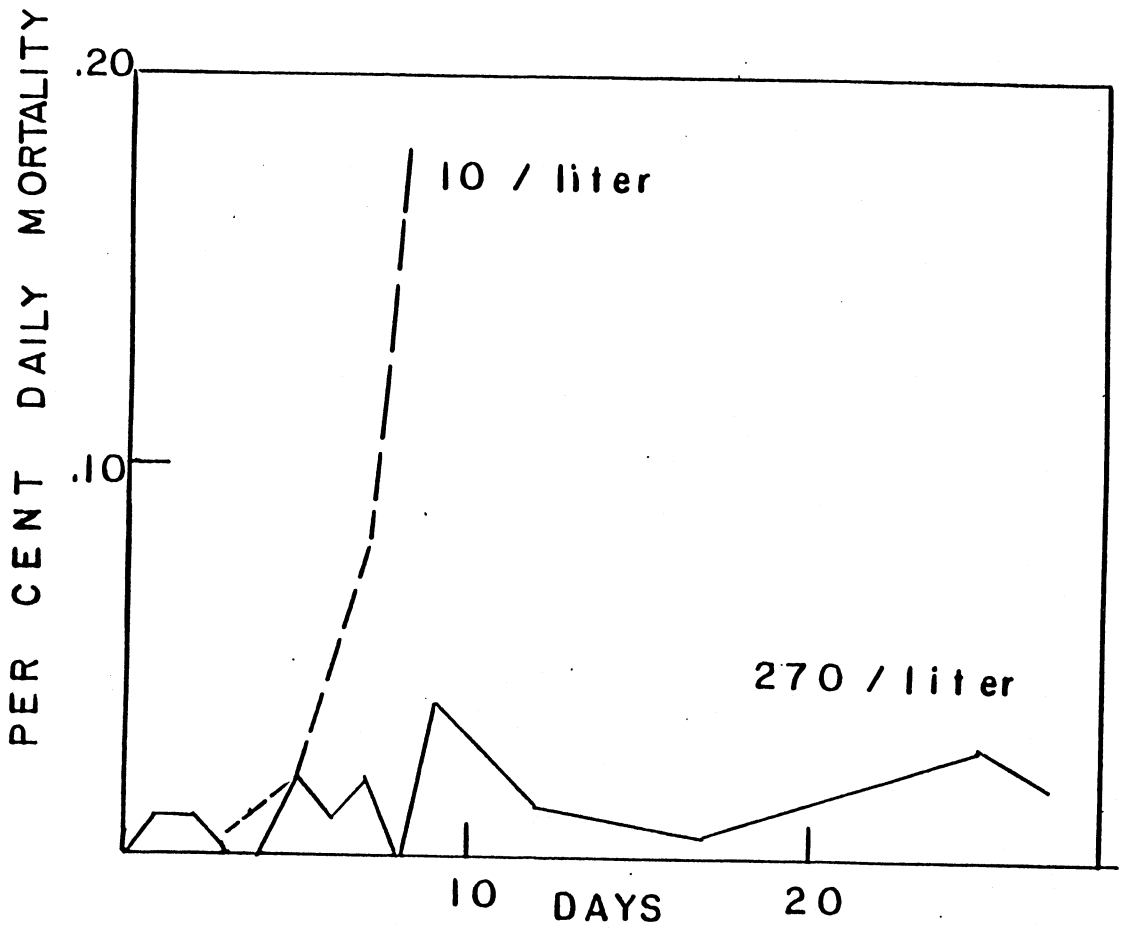
Table 11. Survivorship of parent females in crowded and uncrowded cultures showing number alive on a given date.

Date	270-1	270-2	10-1	10-2	10-3	10-4	10-5	10-6
10/31	54	54	15	15	15	15	15	15
11/1	53	54	15	14	15	15	15	15
11/2	53	54						
11/3	53	54	15	12	15	15	15	15
11/4								
11/5	50	54	15	12	15	15	15	15
11/6	49	54						
11/7	49	53						
11/8	49	53	15	12	14	14	15	15
11/9	49	53						
11/10	48	52	15	10	14	14	14	13
11/11	47	52						
11/12	46	51	13	8	12	12	10	11
11/13	46	51	12	8	12	7	7	7
11/14	43	50						
11/17	42	47						
11/19	42	45						
11/20	42	45						
11/21	42	44						
11/24	39	43						
11/26	36	43						
11/29	32	40						
12/1	32	37						

Table 12. Daily mortality average rates as percent dying per day in low and high density Daphnia cultures.

	Average of 270/liter			Average of 10/l cultures		
Date	Mort.	Surv.	%dying/ day	Mort.	Surv.	%dying/ day
		108			90	
11/1	1	107	.009	1	89	,011
11/2	0	107	0			
11/3	0	107	0	2	87	.011
11/5	3	104	.014	0	87	0
11/6	1	103	.0096			
11/7	1	102	.0097			
11/8	0	102	0	2	85	.0077
11/9	0	102	0			
11/10	2	100	.0196	5	80	.0294
11/11	1	99	.010			
11/12	2	97	.020	14	66	.0875
11/13	0	97	0	12	54	.182
11/14	4	93	.041			
11/17	4	89	.014			
11/19	2	87	.011			
11/21	1	86	.0057			
11/24	4	82	.0155			
11/26	3	79	.0190			
11/29	7	72	.0324			
12/1	3	69	.022			

Figure 3. Daily mortality average rates as percent dying per day in low (10/1) and high (270/1) density Daphnia cultures.


PER CENT DAILY MORTALITY
.20
.10
10 / liter
270 / liter
10
DAYS
20

senescent Chlorella vulgaris compared with no mortality after 10 days in Chlorella harvested from cultures growing in the log-linear growth phase. While the result of increased mortality in the uncrowded cultures here at first may appear to be just an artifact of culture, it may have biological significance. It is possible that Daphnia are sensitive to aging algal cells because of some toxic substance produced by the algae. It is tantalizing to speculate that the algae carried over in the low density cultures are responding to their predators by producing a toxic metabolite, but at present there is no evidence to support this speculation. The complete repression of the feeding rate caused in low density Daphnia by medium preconditioned with crowded Daphnia and the algae they were fed could be interpreted as an effect of the algae. For further discussion of this, see the chapter on the Allelopath Experiment (Chapter 9) and Chapter 10. Part of the art of successful culture of Daphnia is that they not be overfed, i.e., they must be able to clear the water overnight. This assures that there are no aging algae carried over from day to day, but also means that the water is cleared of other microorganisms such as bacteria as well.

Because it appeared that the algal cells were being removed from the cultures more rapidly than anticipated, mortality rates of C. reinhardi in two week old Daphnia medium were measured. At the end of the main experiment (11/13),

all Daphnia were removed from the media of cultures 10-1 to 10-6 and 270-1 and 270-2, and fresh C. reinhardi in log linear growth phase were added by Millipore filter to initial precounted levels of 30,000 - 50,000 cells/ml. Cells were also added to fresh D64 medium as a control. The survival of the algae is shown in Table 13. The mortality rate in the two week old media was very high, and in most cases 97-8% of the cells were dead or abnormal within 24 hours, whereas in the fresh control D64 medium, only 23% died within the first day. The toxic effect was similar in both uncrowded and crowded two week old Daphnia media.

Conductivity measurements were made on the media as well as stock waters and algae media (see Appendix VII for details). Even though the conductivity increased 204 and 403 micromho/cm above that of D64 (1136 micromho/cm), C. reinhardi is tolerant to the highly concentrated HSM medium at 24,383 micromho/cm, so the observed high mortality of the algae is not a simple electrolyte effect.

In order to observe effects on young born into the old Daphnia medium, day old juveniles were removed near the end of the experiment ot fresh medium and food. The juveniles developed very slowly, and showed high mortality in fresh medium. Those that matured enough to produce eggs were small in size and none of their eggs matured. At the end of the observation period, most of the mature individuals had aborted their

Table 13. Survival of C. reinhardi cells in 2 week old unchanged Daphnia medium and in D64 medium over 24 hours.

Sample	% surviving 24 hours	% dying in 24 hours	age of medium days
D64 med	.77	.23	fresh
10-1	.02	.98	14 days
10-2	.06	.94	"
10-3	.11	.89	"
10-4	.03	.97	"
10-5	.02	.98	"
10-6	.025	.975	"
270 aver.	.04	.96	"

eggs. Mortality was heaviest in young taken from the uncrowded cultures, 89% died during the 20 days' observation, compared to 52% mortality in the young from the crowded cultures (see Table 14). The most unusual effect was on egg production: only three of the original 36 young from the uncrowded cultures produced any eggs, and only 13 of the 36 young from the crowded cultures produced eggs. None of these eggs developed completely, and they were aborted within one to five days after being laid. Females without eggs at the end of the study period either exhibited no development of the ovaries, or ovaries contained a gray granular mass or dark abnormal eggs.

In conclusion, crowding of Daphnia pulex in an unchanged medium results in some protection against mortality when compared with uncrowded Daphnia under the same conditions. No reduction of the reproductive rate is seen in crowded, well

Table 14. Effects on young born into old Daphnia medium in low and high density cultures, and removed to fresh medium within one day of birth. 15°.

Density	# of young	days	final L, mm	% mort	# w. eggs	# w. eggs at 20 days	# w. eggs developing
10/1	36	20	1.654	.89	3	1	0
270/1	36	20	1.701	.52	13	1	0

fed Daphnia. The crowded Daphnia had reproductive rates substantially greater than those of uncrowded Daphnia. Aging algae may have repressed reproduction in uncrowded Daphnia. There was induction of males in the crowded but none in the uncrowded populations, indicating that crowding may be a factor determining gamogenesis in Daphnia even when Daphnia are well fed and reproducing well. The mortality of the females in the uncrowded cultures may be related to the age of some of the algae. The repressive effects on juveniles born into the old medium at both low and high density may be related to whatever factor or factors caused the rapid mortality of C. reinhardi in the old Daphnia culture water. Transferring the crowded females to fresh medium and low food caused a depression in the reproductive rate, a huge increase in the percentage of males produced, but no substantial change in the mortality rate.

Chapter 5

Introduction on Feeding Rates

The inherent difficulty in looking for density dependent effects of crowding on sexual and parthenogenic reproduction in Daphnia is that the crowded population, simply by virtue of its numbers, will reduce the food supplies rapidly, so that the amount of food available may limit reproduction. In order to predict the amount of consumption by a particular density of Daphnia during an experimental period, it became necessary to know the rates at which they would feed. In other words, do crowded Daphnia feed at the same rate as uncrowded Daphnia over a wide range of cell concentrations?

Although feeding rates have been actively analyzed in zooplankton, very little research has been carried out on rates at different animal densities. Hayward and Gallup (1976), using D. schödleri, observed a marked depression in filtering rate (F) at densities of 100/liter and 200/liter when compared with 50/liter. The Daphnia were fed the green alga Ankistrodesmus falcatus at a concentration of 10,000 cells/ml. Rough estimates of feeding rates based on their filtering rate figures are: at 50 Daphnia/liter, 15,000 cells $D^{-1}h^{-1}$, at 100 Daphnia/liter, 5,500 cells $D^{-1}h^{-1}$, at 200/liter approximately 4800 cells $D^{-1}h^{-1}$. The average volume of Ankistrodesmus was not reported. An opposite effect of density on feeding rates was reported by Ribi and Cooper

(unpubl., S. Cooper pers. comm.) who observed an accelerated feeding rate at higher densities of D. pulex and D. laevis fed for three minutes on a mixture of yeast at 85 - 90% transmittance and Sephadex five micrometer beads. However, close inspection of their data reveals that D. pulex is feeding at a rate of 2.6×10^5 μm^3 bead volume $D^{-1}h^{-1}$ from 9 Daphnia/liter through 571 Daphnia/liter, and only at the unusually high density of Daphnia of 2286/liter did an increase in rate occur, to 4.6×10^5 μm^3 $D^{-1}h^{-1}$ at night, and to 7.8×10^5 μm^3 $D^{-1}h^{-1}$ during the day. If anything, the rates declined slightly from 9 Daphnia/liter to 571/liter. Cooper felt their work was corroborated by that of Gore (1980), who fed the marine cladoceran Penilia avirostris, in size range 0.5 - 1.1 mm, with plastic micronic beads of mixed sizes at 153,000 beads/ml. The numbers of Penilia ranged from 357 to 9286 per liter. While it is true that the numbers of beads ingested increased as the density increased from 357 Penilia/liter, the volume based feeding rates indicate very depressed feeding overall on the plastic beads. Calculations made indicate the volume feeding rate is equivalent to only 180 cells C. reinhardi $P^{-1}h^{-1}$ at the density of 357/liter, and increases to an equivalent of 3245 cells $P^{-1}h^{-1}$ at the density of 9286/liter.

Studies of feeding rates should be carried out at densities that are comparable to the densities of zooplankton in

natural systems, since one wants to study and explain the mechanisms of phenomena that occur in the real world. In studying feeding rates, it is tempting to overcrowd the animals to achieve rapid reduction in cell (or bead) numbers. Densities of Daphnia above 300/liter are unusual in natural systems on a regular basis. While total populations of cladocera may reach 1000 - 2000/liter in a small pond or 435/liter in a lake, densities are usually lower, e.g., for D. pulex a peak density of 22/liter, for D. longispina 28/liter (Frank 1952, citing Eddy, 1934). Densities of D. pulex reported by students from lakes in the Lake Itasca, Mn. region are: 25.6 /liter, on 7/2/75, and a maximum of 17/liter at 18 meters on 7/25/75 in Squaw Lake (Edwards, Helgen, Sandquist, 1975), 8.4/liter on 6/26/76 in Long Lake (Henry, Huebner, Uhrhammer, 1976), a maximum of 15/liter in Elk Lake (Brown, Duncan and Siders, 1967), 15/liter in Mary Lake (Grothe, 1973), and for Daphnia dubia a maximum density of 60/liter in Elk Lake (Brown, Duncan, and Siders, 1967). Some of the densities I have observed in Square Lake, Washington County, Minnesota are a maximum density of 10.8 D. pulex/liter at 13 m. on 1/23 /77, about 3/liter from three to seven meters on 2/21/77, 3.5 /liter at one meter on 3/14/76. Birge (1896) reported D. hyalina at 331/liter maximum, and at 1170/liter in a surface swarm. Daborn et al (1978), working on aerated sewage ponds in Nova Scotia recorded one maximum density of D. pulex at

750/liter, more typically densities in these enriched waters were 150-300/liter. Slobodkin (1954) in his study on population dynamics in D. obtusa worked with culture densities from 1000 to 14,000/liter, densities far in excess of most natural systems. Gore (1980) does not give the natural densities of Penilia, a marine cladoceran that sometimes dominates the zooplankton in Kingston Harbor, Jamaica, but it is unlikely that the range from 357 to 9286/liter is natural.

The densities chosen for the feeding experiments reported in this research ranged below 300/liter to approximate natural densities and thus reduce the possibility that the results would not apply to a natural system.

In the following chapters, first there will be a general discussion of zooplankton feeding rates and methodology, followed by the experiments on ingestion rates in which a density dependent effect was observed. Second, there follows a discussion of the mathematical functions used to describe feeding rates, followed by the analysis of the data using the Monod and the Ivlev functions. Finally, a combined density dependent feeding function for the feeding rate in Daphnia pulex at various cell levels and Daphnia densities will be proposed.

Mathematics of feeding rates, background

Feeding rates in zooplankton are commonly described as either a feeding or ingestion rate, f, in units of food uptake per animal per time, or as filtering rate, F, in ml water cleared per animal per unit time. Originally it was believed that the filtering rate was constant as food levels were increased, and therefore independent of the food concentration, at least until a saturating level of intake was reached. Species-specific filtering rates were sought in order to characterize the feeding ability of each zooplankter. There was also the hope that each species would show a characteristic maximum feeding rate or f_{max}, with the food level required to reach f_{max} called the "incipient limiting level" (ILL). Figure 4 shows a simplified diagram of the concept.

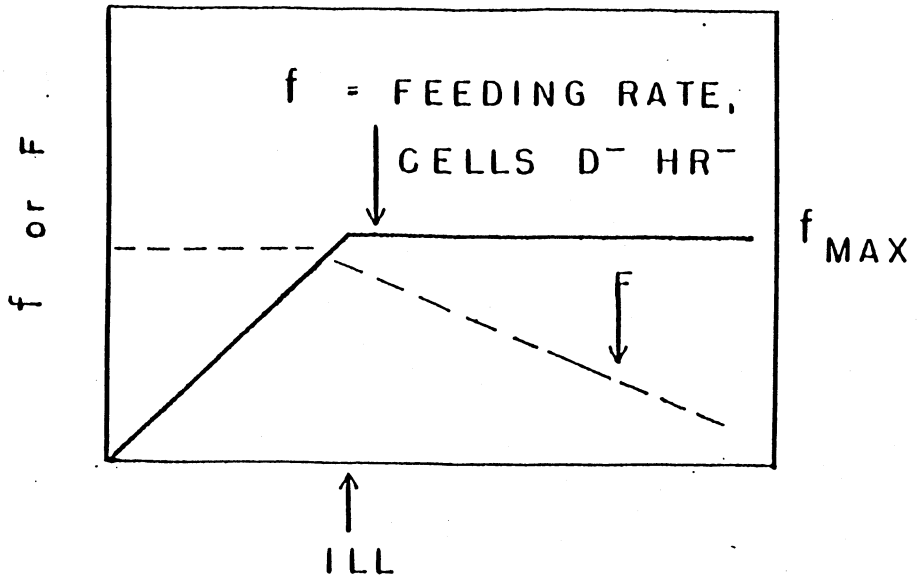
Below the incipient limiting level, then, the feeding rate would depend directly of the cell concentration where F was constant, so

$$f = FC \quad \text{where C = food level, e.g. cells/ml}$$

and $$F = \frac{f}{C}$$

These relationships were observed in Daphnia magna fed yeast (Rigler, 1961), in D. magna fed E. coli, Chlorella vulgaris, yeast and Tetrahymena pyriformis (McMahon and Rigler, 1965), and in D. pulex fed Scenedesmus (Geller, 1975).

Figure 4. Simplified diagram of linear concept of filtering rate (F).



F = FILTERING RATE, ml D^- HR^- = $\frac{f}{C}$

These simple linear equations do not seem to apply to all zooplankton feeding rates. In many cases, the relationship is curvilinear (Burns, 1966; Hayward and Gallup, 1976), with F, the filtering rate exhibiting a negative curvilinear decrease with increasing food. Similar observations have been made in the field (Crowley, 1973), and for animals under starvation conditions (Geller, 1975). Therefore if the filtering rate is not constant then the food level (C) at which the determination was made must be reported for F to be meaningful.

In using the radioactive tracer method a measurement is made of the filtering rate or clearance rate that does not require knowledge of the amount of food available to the animals. When the filtering rate is measured by the radiotracer method of Monakov and Sorokin (1961), where changes in radioactivity/ml in the particultae matter are measured without determining the specific activity of the algal cells, the feeding rate f can be calculated from the given data only if the actual food level is recorded. The use of filtering rates alone to describe feeding is almost meaningless when F rates are determined in natural waters with no measurement of food levels (Burns and Rigler, 1967; Haney, 1971), because of the dependency of F on the food level. In his study on seasonal changes in grazing, Haney (1973) did include measurements of the seasonal changes in suspended particulate

matter. In this method, tracer amounts of ^{32}P labelled yeast cells were added to lake water, and the F rates calculated as

$$F\ (ml\ D^{-1}h^{-1}) = \frac{cpm\ D^{-1}}{\underset{suspension}{cpm\ ml^{-1}}} \quad \frac{60}{\underset{labelled\ food}{mins\ spent\ in}}$$

The assumption was that below the level of "tracer" yeast added, F rates were maximal and independent of cell concentration. Seasonal filtering rates were then compared with those expected in pure yeast alone, and when the field F rates were found to be lower than predicted, the authors concluded that "the prediction that filtering rates of laboratory populations feeding in pure food cultures would not reflect filtering rates in nature" (Burns and Rigler, 1967). Because food levels in the lake water were not measured, however, it is entirely reasonable to assume that the "depression" of F rates from early June on may have been the result of increasing food levels. Because of the ease of measuring F rates, and because of the original concept of constancy, one continues to hear them treated like absolute quantities. This makes valid comparisons of species' and seasonal feeding rates difficult.

The feeding or ingestion rate, f, is conceptually more meaningful in describing feeding in zooplankton because if states directly the amount eaten by a zooplankter/hour, or

f = cells ingested/Daphnia/hr. The feeding rate is strongly dependent on the level of food give, and therefore comparisons between species or seasons again must relate to the same level of food.

Feeding rates also are strongly dependent on body length, usually varying between the square and the cube of the length. Burns (1969) found the filtering rate (most measurements have been made as F rates) was roughly proportional to the square of the body length at 15°, and more proportional to the cube of the length at 20°. The function at 15° was

$$F = .153\ L^{2.16}$$

and at 20°

$$F = .208\ L^{2.80}$$

where L is body length in mm. Chisholm, Stross and Nobbs (1975) similarly found temperature to affect the relationship of F and body length for D. middendorffiana. At 5° they observed

$$F = .458\ L^{1.65}$$

and at 11°

$$F = .458\ L^{3.17}$$

for this Arctic species. Geller (1975) measured ingestion rates in D. pulex at 15°, and found a relationship above the

incipient limiting level of

$$I = .41\left(\frac{L}{2}\right)^{1.82}$$

where I is the ingestion rate as $\mu g\ C\ D^{-1}h^{-1}$, and for food levels below the incipient limiting level

$$I = I_{2mm}\left(\frac{L}{2}\right)^{2.42}$$

At the higher food levels, he observed a flattening out of the size dependency curve, that is a reduction in the size of the exponent. When he used body weight instead of body length, he found there was more ingestion per microgram body weight for smaller Daphnia than for larger animals.

Filtering rates are dependent on temperature as well as oxygen levels. Hayward and Gallup (1976) observed with D. schödleri an inverse parabola of F and temperature, with optimum F near 20°, a small decrease to either side to 15° or 25°, and sharper decrease with temperature from 10° to 5°. As described in the Introduction, dissolved oxygen does not affect filtering rates until it drops below 2-3 mg/liter. For D. pulex there is a steep decline in F below 3 mg/liter. (Kring and O'Brien, 1976). In D. magna there is a similar decline in F below O_2 of 2.5 mg/l, but interestingly, F declines linearly at all levels of O_2 in D. galeata mendotae (Heisey and Porter, 1977). This tolerence of lower oxygen in

D. pulex combined with its ability to synthesize hemoglobin adaptively explains in part the well known ability of this species to inhabit the hypolimnion in stratified lakes.

A real difficulty in comparing feeding rates in zooplankton results from the diverse way in which food levels are measured and described. Some examples are given below:

cells $D^{-1}h^{-1}$	Ryther, 1954 Hayward and Gallup, 1976
calories, mg dry wt, cells	Richman, 1958
chlorophyll a $liter^{-1}$	Vivjerberg, 1976
Klett units	Arnold, 1971, Smith, 1963, Hall, 1964, Slobodkin, 1954, Weglenska, 1971
mg $liter^{-1}$ wet wt.	Weglenska, 1971
μ g C $Daphnia^{-1}h^{-1}$	Geller, 1975
μ m^3 $Daphnia^{-1}h^{-1}$	Geller, 1975
joules $D^{-1}h^{-1}$	Hrbáckova and Hrbácek, 1978
transmittance (of yeast) plus bead #	Cooper, pers. comm.

Obviously there is a real need for some commonality of units so that results can be compared. Since Daphnia may be feeding on edible algae on a volume basis, this should always be reported, and will be discussed later.

Descriptions of food levels such as chlorophyll, joules and carbon may be meaningful as indicators of nutritional

value and assimilation rates where actual incorporation of food is measured, but are less useful in indicating the mass of particulate matter ingested because of variation in cellular content, e.g., a reduction in chlorophyll content may be the broadest response of algae to nutrient deficiency (Healey, 1973), with chlorophyll above 10 μg/mg dry wt. considered as no deficiency, and below 5 μg/mg as a severe deficiency (Healey, 1978). Chlorophyll per mg dry weight varies with the algal species: 46 mg for C. pyrenoidosa and 8 mg for Cyclotella cryptica (Healey, 1973), as well as with the conditions, particularly light (Fogg, 1965). The use of joules/unit time was derived from cell nitrogen content which also would be expected to vary depending on cell condition: protein content drops 3 - 7 fold in N nutrient deficient cells of C. pyrenoidosa, and N content drops 7 -fold (Healey, 1973).

Carbon/algal volume ranges about 20-fold for various species of algae (Mullin, Sloan and Eppley, 1966), so carbon as a measure of food may not provide a meaningful comparison of ingestion rates among zooplankters feeding on different species of algae, if the rates are volume-dependent. Cell carbon per unit volume decreases as the cell volume increases, so smaller algae have a higher proportional carbon content. A regression of cell carbon to cell volume for 14 species of algae is

$$\log C = .76 \log V - .29$$

(Mullin, Sloan and Eppley, 1966). Approximate values from this regression illustrate the changes in C with volume:

V, μm^3	C, carbon	C/V
100	64.6	.65
1000	371.6	.37
10000	2138	.21

Within a single species, however, C content changes much less in nutrient deficient cells. The C content of C. pyrenoidosa increases slightly (by 1.25x) in N deficiency, and decreases slightly (to .94x) in P deficient Anacystis nidulans (Healey, 1973). Hence, cell carbon can be used for ingestion rates with single species in addition to its usefulness in assimilation rate studies (e.g., Lampert, 1977) and in the recent work on determinations for threshold levels for reproduction (Lampert and Schober, 1980, A. Duncan, pers. comm.)

Ingestion rates in Daphnia pulex were fairly independent of the algal species when food was measured by volume of food cells, with separate equations for algae 3 - 15 micrometers diameter and for 20 - 50 micrometer algae (Geller, 1975). Daphnia feeding on six species of algae showed similar ingestion volumes per unit time (see list ahead). The list also

shows Geller's calculations for volume-based rates from the data of other researchers. Variability among rates appeared when carbon content was the measure of food: minimum to maximum rates ranged from unity to 8.4. The right hand column shows the variation in cell volume on a per unit carbon basis over a 16-fold range.

Ingestion volumes 2 mm Daphnia x 10^6 μm^3 h^{-1}	Alga	Aver cell vol μm^3	Vol/C content x 10^6 μm^3/gC
2.1	Scenedesmus	111	5.22
1.7	Stichococcus	2.1	2.5
3.3	Staurastrum	40,000	40
4.4	Nitzschia	360	14.8
4.9	Asterionella	190	12.5
1.5	Stephanodiscus	250	9
5.2	Chlorella	(from Kersting and Van der Leeuw, 1974)	
1.5	E. coli		
5.1	C. vulgaris	(last 3 from data of McMahon and Rigler, 1965, D. magna)	
4.8	Saccaromyces		

Volume based feeding rates derived by Geller (1975) for 2 mm D. pulex at 15° were around 1.8 x 10^6 $\mu m^3 D^{-1} h^{-1}$ for smaller cells (3 -15 μm^3 diameter) and 4.2 x 10^6 $\mu m^3 D^{-1} h^{-1}$ for larger cells. The "incipient limiting level" of food by volume ranged from 1 - 5 x 10^6 μm^3/ml, below which Daphnia fed at volume rates of 1.7 x 10^6 $\mu m^3 D^{-1} h^{-1}$ and lower. In

marine copepods, volume-based feeding rates ranged from 0.1 to 1.5 x 10^6 μm^3 $copepod^{-1}h^{-1}$ (Mayzaud and Poulet, 1978), with rates of 0.16 - 0.9 x 10^6 $\mu m^3 copepod^{-1}h^{-1}$ at the naturally low particle levels of the Bedford Basin of 0.8 - 4.8 x 10^6 μm^3/ml (Poulet, 1973).

Measurement of algal volume in single species of simple geometry is relatively convenient if the division cycle of the alga is understood, but measurement of natural mixed assemblages is more tedious and is not usually reported. Typical volumes are far too minute for the microhematocrit: over 4 x 10^6 cells of C. reinhardi would be needed to fill a 5 mm length. The volume of algae in a ml of heavy stock culture at 1.5 x 10^6 cells/ml is only 4 x 10^{-4} cc.

Volume changes of mixed species can be measured with the Coulter Counter with a wide range of threshold settings to cover all the cell volumes (Kersting and Holterman, 1973; Parsons, 1965), because the magnitude of the voltage pulse produced as the particle flows through the aperture is proportional to the cell volume (Maloney, Donovan and Robinson,1962). This would be extremely useful even if the individual species' volumes could not be separated, as long as the size range is appropriate for the zooplankter. The disadvantages of the Coulter Counter method are that non-algal particulate matter such as fecal debris, detritus, and non-organic particles will be measured, and that at very low cell concentrations there

may be problems of precision and accuracy (Mullin, Stewart and Fuglister, 1975). Particle size selectivity in marine copepods has been effectively studied using the Coulter Counter (Richman, 1981).

Feeding rates have been measured by cell count, radiotracer and volume-Coulter Counter methods. The cell count method is tedious but direct. Cell levels are measured at regular intervals, the average cell concentration as the interval food level is computed from the log rather than the arithmetic average because cell removal is exponential. Changes in cell concentration over the interval are kept low, around 20%. The feeding rate is calculated as

$$f = \frac{(C_2 - C_1)\ 1000\ ml}{D\ liter^{-1} hours} = \text{cells eaten } D^{-1} h^{-1}$$

at an average cell concentration for the interval of

$$C = \text{antiln}\left(\frac{\ln C_1 + \ln C_2}{2}\right) \text{ or } = e^{\left(\frac{\ln C_1 + \ln C_2}{2}\right)}$$

The three basic radiotracer methods are: 1) radioactivity of the particulate matter is measured before and after feeding, the method of Monakov and Sorokin (1961), 2) activity of the algal suspension is measured at t_o but not at t_t, instead the radioactivity in the animals at the end of the feeding interval is measured, the method of Marshall and Orr (1955), and method 3) is the same as 2), except that the

feeding interval is kept shorter than the estimated gut passage time, usually a few minutes, to avoid fecal and assimilatory loss of activity, the method of Nauwerck (1959). The advantage of the last method over the cell count method is that changes in cell levels can be kept very small, so the measurement is closer to an instantaneous rate. The disadvantages include the possibility that some assimilation may occur, that the animals must behave "normally" the instant they are transferred to the radioactive cells, that a particular population cannot be observed over a period of time, and the animals must be killed at the end of the short interval.

Chapter 6

Experiments on Feeding Rates in *D. pulex*

For the feeding rate experiments described below, the cell count method was used. A description of the method is presented in Chapter 2.

In looking for effects of density on feeding rates, the average rates of populations of *D. pulex* were measured rather than those of isolated individuals. Inclusion of social interaction and other possible effects of crowding, as well as the inclusion of genetic variability was desired in these experiments. Measurement of feeding rates in a population means that a rectilinear feeding response to higher food levels could likely be averaged into a curvilinear response because of individual variability in feeding rates. Studying feeding rates in isolated *Daphnia* at various densities not only means placing single *Daphnia* in 3.3 ml for the density of 300/liter, but also eliminates natural interactions between individuals that could affect feeding in a population.

Because starved *Daphnia* feed at a rate higher than well fed *Daphnia*, and also to allow the stock *Daphnia* to adjust to the change in medium, the *Daphnia* were preincubated overnight (OVPI) at each experimental food level. The next morning, the *Daphnia* were transferred to another jar at the same level for a one hour preincubation (PI) just before transfer to the medium for the actual measurements (I, incubation). A simplified flow chart for these experiments is given on p. 93.

Feeding experiments

Preconditioned *Daphnia* at density

|

Overnight incubated
at each food level -- OVPI

|

AM, preincubation -- PI
at each food level, 1 hr
Algae dark adapted

|

AM, incubation I,
0 - 12 hrs subsampling
Algae dark adapted

The Daphnia chosen for the study were of a similar size, and were measured at the end of the experiments. The feeding tests were carried out at a temperature between 15.0 - 15.5° C. Jars were stirred regularly and gently in a routine way to prevent migration of the algae to the surface. The tests were carried out in the dark.

The t_o or initial subsample of the cultures was taken just before the Daphnia were added to the I jars. Typically the cultures would be subsampled over 12 hours, although the time of subsampling varied, with appropriate subsampling at intervals depending on the density of the Daphnia, and their expected ingestion rates. The aliquots, usually 11 ml, were removed and preserved for cell counts with I/KI solution, and at the same time, the volume of the medium was reduced if necessary and Daphnia were removed to preserve the original density of the Daphnia. All Daphnia were preserved in formalin-sucrose preservative for measurement later.

For most of the experiments, particle free medium, obtained by sterile Millipore filtration through a 0.2 micrometer prerinsed Gelman Metricel filter, was used for the PI and I incubations. Glassware was either autoclaved or rinsed with alcohol, dried, and then rinsed with sterile glass distilled water followed by a particle free water rinse. Preliminary experiments indicated that the variability was reduced when particle free medium was used.

It was discovered that C. reinhardi, even when transferred to the dilute D64 Daphnia medium, could double in number during the course of several hours' subsampling, so the algae had to be placed in the appropriate Daphnia medium at the desired levels overnight in the dark for use in the PI and I incubations the next day. Algae treated in this way were usually quite stable through the period of subsampling, particularly when NH_4Cl at 0.005 g/liter was added to the D64, with very little mortality occurring in the control jars without Daphnia. Control jars of algae at each food level were subsampled usually at the same intervals as the feeding I jars. One advantage of this cell count method over the radiotracer methods is that the subsampling generates data on feeding rates of the population at several cell levels as the food is consumed.

The basic interval feeding rate, f, as cells consumed $D^{-1}h^{-1}$ is calculated as follows:

$$f = \frac{(C_t - C_o)\ 1000\ ml}{h_i\ N}$$

where $C_t - C_o$ is the change in the cell concentration over the interval, N is the number of Daphnia per liter, $\frac{1000}{N}$ the volume in ml per Daphnia, and h_i the time of the interval in hours. The filtering rate F, then, as ml $D^{-1}h^{-1}$, is obtained as follows:

$$F = \frac{f}{C_A}$$

where C_A, or the interval averaged cell concentration, is

$$C_A = \text{antiln} \left(\frac{\ln C_o + \ln C_t}{2} \right)$$

One of the early measurements of feeding rates was a simple comparison of the feeding rates of two stock of D. pulex with that of D. magna. Daphnia at 50/liter were preincubated overnight in algae resuspended from Millipore filters in autoclaved tap water made up with minimal medium (MM, see Appendix VIII). In the morning, the Daphnia were preincubated 35 minutes in algae that had been dark adapted overnight in the same medium and then transferred to I jars for the six hour incubation. Volumes are listed below.

Daphnia	# of Daphnia	Vol,ml	PI,mins	I, hrs
D. pulex, Square Lake	15	300	35	6
D. pulex,stock	15	300	35	6
D. magna	10	200	35	6

Since there was no control jar of algae in this early experiment, it was assumed that there was no change in the numbers of algal cells other than those consumed by Daphnia.

If the algae were multiplying, the feeding rates would be underestimated, if they were dying, the feeding rates would be overestimated because loss by cell death would be counted as uptake. The results are given below:

	t_6, cells/ml	f, ingestion rate
t_o average	26614	
D. pulex, Square Lake	23619	9983
D. pulex, stock	23600	10452
D. magna	4656	73193

The feeding rates reported here can be compared with those measured by Geller (1975) for D. pulex at 50/liter and at 15°, by using the average measured volume for C. reinhardi of 288 μm^3/cell.

	Ingestion as $\mu m^3 \times 10^6 D^{-1} h^{-1}$		body length mm
	measured	Geller	
D. pulex, Square Lake	3.171	2.681	2.51
D. pulex, stock	2.936	2.233	2.26
D. magna	22.21	4.155	3.21

From feeding rate measurements using D. pulex at 50/liter, Geller (1975) developed the following equation to predict

ingestion on a volume basis for small (3 - 15 micrometer diameter) food cells at 15°

$$I_{vol} = 1.8 \left(\frac{L}{2}\right)^{1.77} \quad (10^6 \ \mu m^3 \ D^{-1}h^{-1})$$

In both cases the D. pulex stocks are feeding at ingestion volumes somewhat higher than those predicted by Geller. The equation does not fit feeding in D. magna, whose feeding rate was five times greater than that expected for D. pulex of a similar size. The calculated ingestion volume of 17×10^6 $\mu m^3 \ D^{-1}h^{-1}$ based on the data of McMahon and Rigler (1965) for D. magna feeding on Chlorella at a rate of 500,000 cells $D^{-1}h^{-1}$ is close to the feeding volume observed here for D. magna of 22.2×10^6 $\mu m^3 D^{-1}h^{-1}$, assuming the volume of the cells to be 34 μm^3/cell. However, Porter and Orcutt (1980) observed a maximum rate of only 6.39×10^6 $\mu m^3 \ D^{-1}h^{-1}$ in 2.66 mm D. magna. The smaller size of their D. magna accounts for some of the reduction in their rates.

In the next two feeding experiments at 50 Daphnia/liter, feeding rates were lower than those obtained in several subsequent experiments. For these two experiments, NH_4Cl was added at 0.05 g/l to the medium to maintain the algae in good condition and to prevent clumping. Sueoka (1960) had determined that this was the minimal level of NH_4Cl required in the minimal medium, MM, for maintainance but not growth of C. reinhardi . In the later experiments (Experiments 1 to 5,

following), NH_4Cl was either omitted or used at 0.005 g/l and feeding rates were much higher. Clumping of the cells was very rare in these experiments.

In the first of the 50 Daphnia/liter experiments, feeding was actually inhibited at algal cell concentrations above 100,000 cells/ml. In the second run, seven different levels of algae alone and of algae plus Daphnia were preincubated overnight in the dark. Daphnia were transferred to a one hour preincubation (PI) in dark-adapted algae for the six hour feeding incubation. At the beginning of the experiment, replicate precounts were taken at each food level. At the end of the six hour period, replicated aliquots were taken from each of the seven levels' control jars and experimental Daphnia feeding jars. Incubations were run with 200 ml in all jars, 10 Daphnia in each experimental jar. The results are presented in Table 1.

Table 1. Feeding rates in Daphnia at 50/liter, 15°.

Jar	Aver. C_o	f, cells $D^{-1}h^{-1}$	F, ml $D^{-1}h^{-1}$
1	4219	6155	1.89
2	7355	7471	1.19
3	11791	9627	.98
4	22225	9081	.45
5	40627	20969	.56
6	97602	30889	.33
7	135007	31643	.24

A calculated ln ln regression using all food levels with ln f as y values, ln C_A as x values gives the equation

$$\ln f = .4819 \ln C + 4.714$$

or, in the exponential form:

$$f = 111.53\ C^{.4819}$$

Under these conditions the feeding rate of the Daphnia is varying approximately with the square root of the cell concentration. The slope of the line, .48, indicates the rate of change of f in relation to the cell concentration. Obviously, if the filtering rate F were actually constant, which it is not, the feeding rate would increase linearly until saturation, and the exponent of C would be 1. A plot of this data is shown in Figure 5 . The curvilinear nature of the relation between f and food levels is clearly seen in the linearity of the ln ln transformation of data from another early experiment presented in Figure 6.

Experiment 1. Feeding rates of Daphnia at 30/liter.

For this experiment, Millipore-filtered D64 medium at half strength was used, and NH_4Cl added at 1/10th the minimal level, to .005 g/liter to prevent mortality of the algae. Six food levels were used with replicates, and a control jar was run at each food level. Daphnia were preincubated overnight and for one hour before the incubation at each food

level, and the algae were dark adapted in the medium overnight for use the next day in the preincubation (PI) and the incubation (I). Volumes and sampling times are given in Table 2. Under these conditions the algae in the controls were quite stable, and the control counts over time for the six food levels are given in Table 3. The Daphnia were measured at the end of the experiment and the average sizes ranged from 2.19 to 2.33 mm (Table 4).

Table 4. Average size of Daphnia used in Experiment 1. 30/1.

Jar	$\bar{X}$, mm	S.D.	Jar	$\bar{X}$, mm	S.D.
1a	2.19	.126	4a	2.19	.119
1b	2.26	.177	4b	2.25	.140
2a	2.22	.168	5a	2.33	.160
2b	2.21	.127	5b	2.29	.127
3a	2.26	.123	6a	2.22	.190
3b	2.27	.127	6b	2.24	.140

The feeding rates were calculated as described previously in the methods, using the ln average cell concentration of cell levels at the beginning and end of the interval (Table 5). These values are used later for analysis of feeding rate functions.

Figure 5. Feeding rates in preliminary measurements of Daphnia at 50/liter, 15°. Filtering rate, F, is the slashed line.

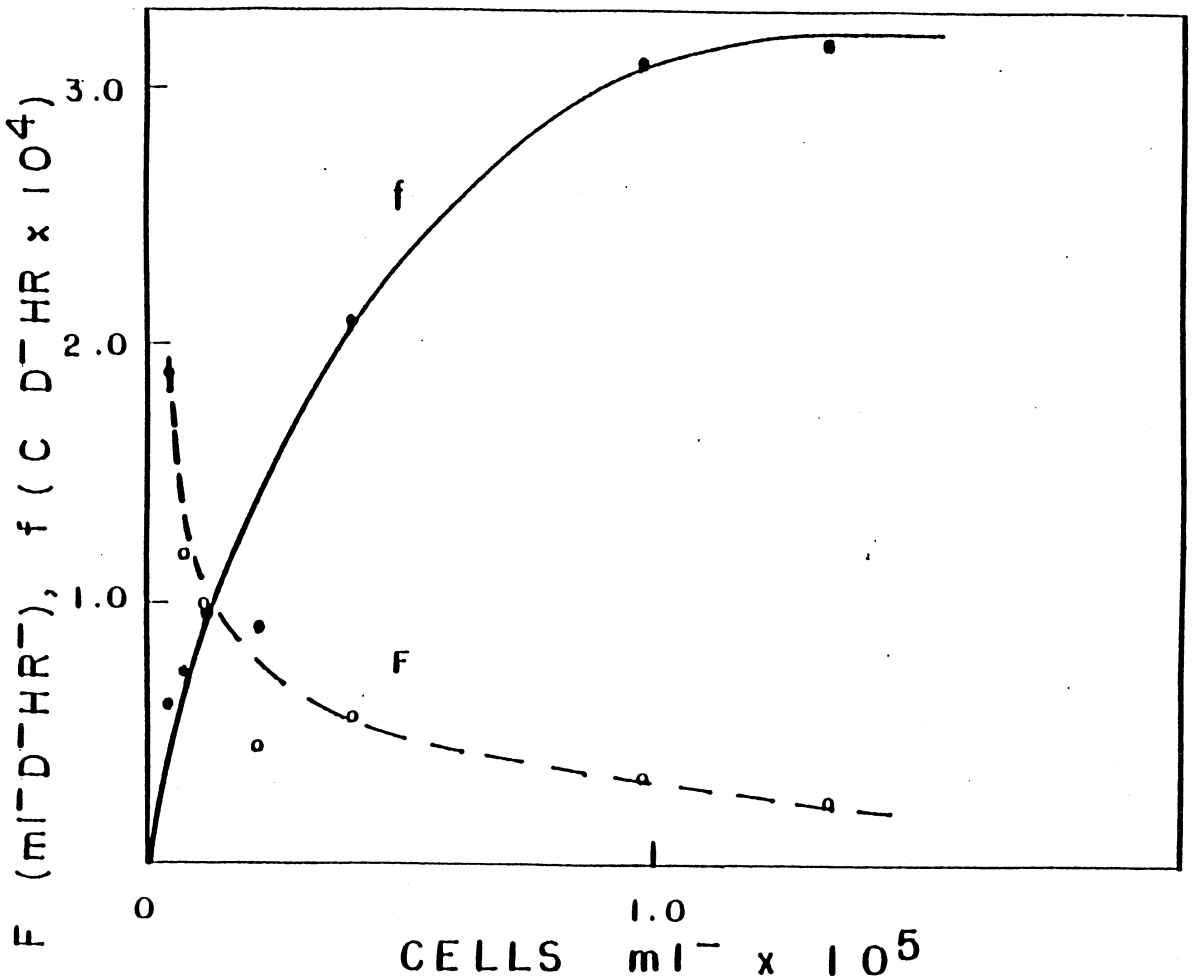

F (ml⁻D⁻HR⁻), f (C D⁻HR x 10⁴)
3.0
2.0
1.0
0
f
F
1.0
CELLS ml⁻ x 10⁵

Figure 6. Transformation of preliminary feeding rate data on ln ln basis to show the curvilinear nature of the feeding rate.

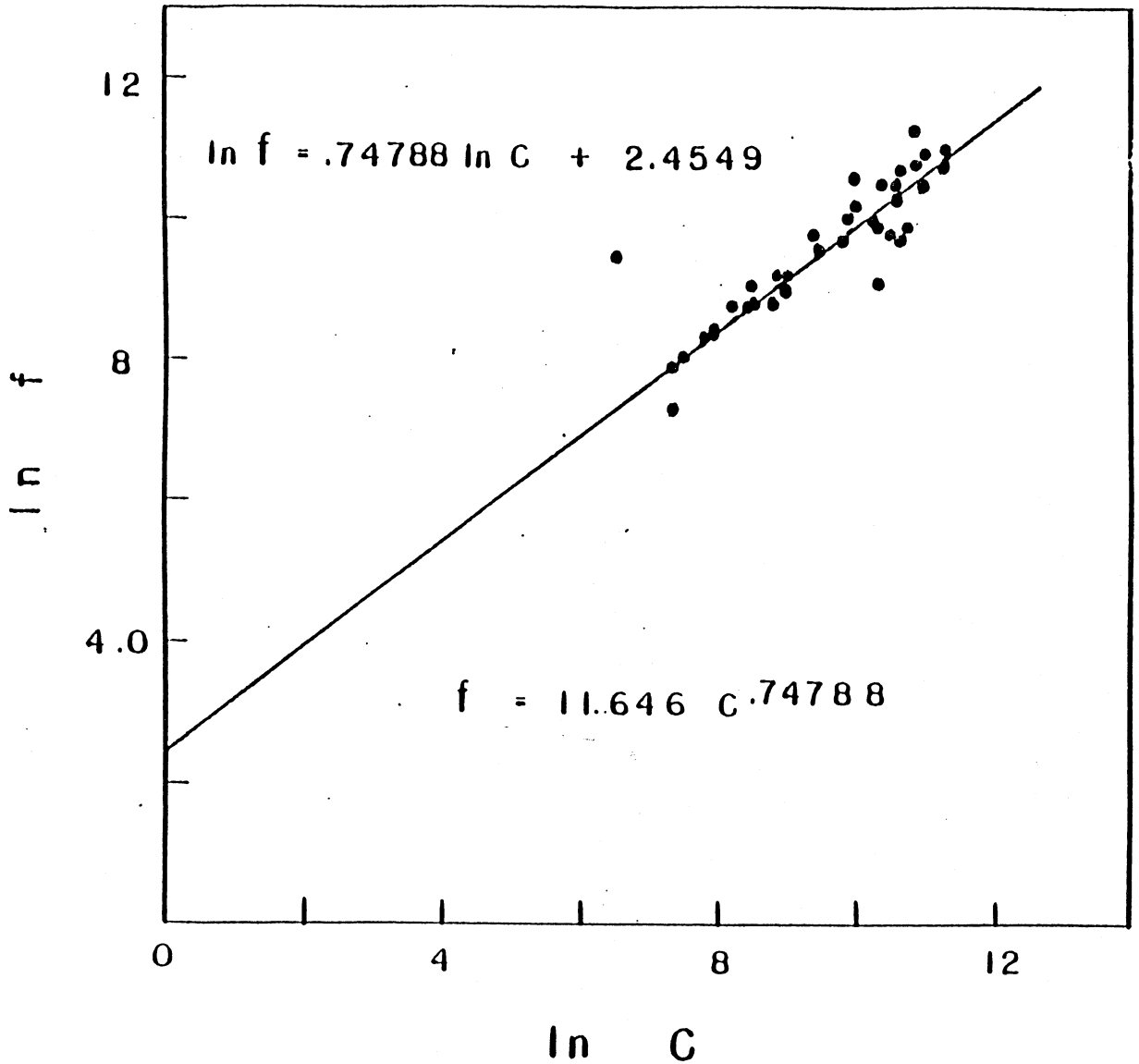

12
8
4.0
ln f = .74788 ln C + 2.4549
f = 11.646 C.74788
0
4
8
12
ln f
ln C

Table 2. Experiment 1. *Daphnia* at 30/liter, 15°, volumes and sampling times.

Exptl jars	# D/jar	Control	Jar vol,ml	Sampling times,hrs
1a, 1b	15	1c	500	0, 4, 6, 8, 10, 12
2a, 2b	15	2c	500	0, 6, 12
3a, 3b	15	3c	500	0, 12
4a, 4b	15	4c	500	0, 12
5a, 5b	15	5c	500	0, 12
6a, 6b	15	6c	500	0, 12

Table 3. Some of the control levels of algae over time dur- Experiment 1, in cells/ml.

	Sampling time, hours				
Level	0	4	6	8	12
1	7459	7504	6645	7594	7353
2	19136		17479		20266
3	33338				33488
4	54583				47840
5	74247				76996
6	98845				101105
7	116775				116700

Table 5. Results of Experiment 1 at 30 Daphnia/liter. Calculated f rates and interval ln averaged cell levels, C_A.

Sampling interval, hrs	Jar	C_A	f
0 - 6	1a	6926.9	6025.2
0 - 6	1b	5932.3	10985
6 - 12	1a	5195.7	12991
6 - 12	1b	4359.2	7663
0 - 6	2a	17939	24313
0 - 6	2b	15398	12356
6 - 12	2a	14582	22048
6 - 12	2b	12603	26682
0 - 12	3a	34378	40494
0 - 12	3b	29446	25739
0 - 12	4b	43059	56689
0 - 12	5a	77051	56083
0 - 12	5b	68862	81719
0 - 12	6a	94098	64944
0 - 12	6b	87123	78583
0 - 12	7a	96802	131686
0 - 12	7b	103962	101847

Experiment 2. Feeding rates of Daphnia at 30/liter.

For this experiment, the same procedure was used as outlined for Experiment 1, except there were seven initial food levels. Average initial cell concentrations and the sampling intervals are provided in Table 6. The Daphnia were preincubated in fresh medium and food for one additional day prior to the overnight preincubation. Body size measurements made before the AM preincubation are presented in Table 7.

The feeding rates and the interval ln averaged C values are given in Table 8. There were changes in the number of algal cells in the controls, in particular some mortality (15%, 19%) occurred in the two highest concentrations of algae. Corrections were made in the calculations to compensate for these changes. The algae were stable at other cell concentrations.

Experiment 3. Feeding rates of Daphnia at 90/liter.

In this experiment, there was no addition of NH_4Cl, and regular full strength D64 was used. Each experimental jar contained 200 ml medium and 18 Daphnia. Average C_o levels and the interval sampling hours are presented in Table 9. The one hour preincubation was carried out only for the first three food levels, and it was assumed that the cell levels in jars 4, 5 and 6 would not be diminished very much overnight.

Table 6. Experiment 2. Density 30/liter. Initial average cell concentrations and subsampling intervals.

Jars (c = control)	C_o	Subsampling interval hours
1 a, b, c	4721	0, 1.5, 3, 4.5, 6
2 a, b, c	10149	0, 3, 6, 9, 12
3 a, b, c	20725	0, 6, 12
4 a, b, c	29232	0, 6, 12
5 a, b, c	37268	0, 12
6 a, b, c	58990	0, 12
7 a, b, c	120467	0, 12

Table 7. Size of Daphnia used in Experiment 2. Samples a and b are pooled.

Jars (a, b)	$\bar{X}$, mm	S.D.
1	2.27	.145
2	2.25	.129
3	2.26	.240
4	2.34	.104
5	2.27	.175
6	2.31	.124
7	2.16	.163

Table 8. Experiment 2. 30/1. Calculated feeding rates (f) and interval cell concentrations (C).

Hrs Sampling interval	Jar	C	f
0 - 3	1a	4701.6	13617
3 - 6	1a	3839.7	6194.4
0 - 6	1b	3080.1	8419
0 - 6	2a	8997.7	22434
0 - 6	2b	6890.9	21179
6 - 9	2a	6163.1	21430
6 - 9	2b	4178.7	21262
9 - 12	2a	3739.7	29131
9 - 12	2b	2547.5	15347
0 - 6	3a	19598	57344
0 - 6	3b	14283	18278
6 - 12	3a	9361.0	51691
6 - 12	3b	5256.1	58681
12- 24	3a	3353.6	10729
12- 24	3b	2072.2	530.3
0 - 6	4a	32396	32439
0 - 6	4b	18682	43950
6 - 12	4a	15593	118867
6 - 12	4b	9089.5	53530
12- 24	4a	6243.2	9626.4
12- 24	4b	3445.0	9166.1
0 - 12	5a	24341	100417
0 - 12	5b	12755	55333
12- 24	5a	9813.1	12160
12- 24	5b	4062.4	9919.7
0 - 12	6a	37492	96789
0 - 12	6b	35058	106833
0 - 12	7a	100150	151933
0 - 12	7b	65324	196928

Table 9. Experiment 3. 90/1. Initial average cell concentration (C_o) and subsampling intervals in hours. Controls were sampled at 0, 6, and 12 hours

Jars	C_o	Interval sampling hours
1 a,b,c	5990	0, 2, 4, 6, 8, 10, 12
2 a,b,c	9194	"
3 a,b,c	21740	"
4 a,b,c	45995	"
5 a,b,c	62217	"
6 a,b,c	85924	"

Sampling was carried out every two hours, and the calculated values were based on four hour intervals, since the two hour intervals were too frequent for adequate changes in cell numbers. The D. pulex stock was collected from Square Lake a few days before the experiment, and kept under standard stock conditions as described previously. The body lengths of the preserved Daphnia were measured at the end of the experiment (Table 10). These Daphnia averaged 0.3 mm larger than those used in the other experiments. Geller's formula for ingestion volume for D. pulex feeding on 3 - 15 micrometer diameter cells

$$I_{vol} = 1.8 \left(\frac{L}{2}\right)^{1.77} \qquad (10^6 \ \mu m^3)$$

predicts that a 2.2 mm Daphnia will feed at a rate approximately 20% lower than a 2.5 mm Daphnia.

The calculated feeding rate values for four hour intervals and the ln averaged interval C_A values are given in Table 11. Because there was variable mortality in the control algae, appropriate corrections were made. Feeding rates were much higher than those predicted by Geller (1975) for either 2.2 mm Daphnia , 7399 cells $D^{-1}h^{-1}$, or for 2.5 mm Daphnia, 9278 cells $D^{-1}h^{-1}$ feeding on C. reinhardi cells.

Experiment 4. Feeding rates of Daphnia at 270/liter.

In this experiment, the same media and procedures were used as in Experiments 1 and 2. Initial C_o values and

Table 10. Size of Daphnia used in Experiment 3. 90/1.

Jar	$\bar{X}$, mm	S.D.
1a	2.530	.2151
1b	2.489	.1998
2a	2.559	.1500
2b	2.457	.1652
3a	2.559	.1021
3b	2.454	.2234
4a	2.562	.1368
4b	2.473	.2753
5a	2.440	.2066
5b	2.494	.1593
6a	2.569	.1784
6b	2.542	.0897

Table 11. Experiment 3. 90/1._ Calculated feeding rates (f) and interval cell concentrations (C).

		C	f
1a	0 - 4	4679.5	6313.1
1b	0 - 4	4594.4	8685
2a	0 - 4	6583.4	13219
2b	0 - 4	7693.2	10080
3a	0 - 4	19027	22741
3b	0 - 4	17895	16550
4a	0 - 4	38038	35575
4b	0 - 4	40088	31078
5a	0 - 4	52177	48742
5b	0 - 4	50774	81618
6a	0 - 4	74477	61736
6b	0 - 4	78360	46458
1a	4 - 8	2705.1	4694.7
1b	4 - 8	2423.6	4178.6
2a	4 - 8	3322.0	6201.7
2b	4 - 8	4783.2	6480.6
3a	4 - 8	12518	14369
3b	4 - 8	11528	17771
4a	4 - 8	30527	8894.4
4b	4 - 8	30694	21869
5a	4 - 8	41750	16114
5b	4 - 8	35780	17472
6a	4 - 8	57303	36206
1a	8 - 12	1411.7	2741.4
1b	8 - 12	1498.8	1464.7
2a	8 - 12	1723.0	3180.8
2b	8 - 12	2784.8	4701.7
3a	8 - 12	8658.6	7896.7
3b	8 - 12	6759.7	9863.6

(Table 11 continues on the next page)

Table 11, continued

Jar	Hours Sampling Interval	C	f
4a	8 - 12	20709	39344
4b	8 - 12	21657	26786
5a	8 - 12	31679	36622
5b	8 - 12	28588	21767
6a	8 - 12	42059	46039
6b	8 - 12	45930	19881

Table 12. Experiment 4. 270/1. Initial average cell concentrations (C_o) and subsampling intervals in hours.

Jar	C_o	Subsampling intervals
1a	123142	0, 2, 4, 6, 8, 10, 12
1b	108262	"
2a	91461	"
2b	87017	"
3a	59442	"
3b	56806	"

Table 13. Size of Daphnia used in Experiment 4.

Jar	$\bar{X}$, mm	S.D.
1a	2.37	.173
1b	2.34	.127
2a	2.38	.173
2b	2.35	.170
3a	2.39	.205
3b	2.36	.112

Table 14. Experiment 4. 270/1. Calculated feeding rates (f) and interval cell concentrations (C).

Jar	Hrs Sampling Interval	C	f
1a	0 - 4	101113	37146
1b	0 - 4	86460	36310
2a	0 - 4	64138	43041
2b	0 - 4	63316	37914
3a	0 - 4	31269	39809
3b	0 - 4	31511	36414
1a	4 - 8	62073	33903
1b	4 - 8	59300	16777
2a	4 - 8	30619	22345
2b	4 - 8	30970	23621
3a	4 - 8	6341.8	12967
3b	4 - 8	7714.7	13032
1a	8 - 12	27839	27509
1b	8 - 12	32226	28275
2a	8 - 12	9923.1	14926
2b	8 - 12	8947.0	15717
3a	8 - 12	1335.3	1589
3b	8 - 12	1806.4	2266

subsampling intervals are in Table 12. The incubation jars a and b contained 70 Daphnia in 260 ml algae and medium at each food level. The lengths of preserved Daphnia measured at the end of the experiment are given in Table 13. The control algae were stable over the 12 hour period. For analysis the four hour subsampling intervals were used to calculate the feeding rates. Feeding rates and C_A values are presented in Table 14.

Experiment 5. Feeding rates of Daphnia at 270/liter.

The procedures and media were similar to those in Experiment 4, except that the vitamins and growth factors were omitted from the ½D64 medium. The initial C_o values and the subsampling intervals are given in Table 15. The incubation jars a and b contained 68 Daphnia each in 251 ml initially, and the control c jars had 251 ml volume at each food level. Daphnia in this experiment were carrying the parasitic fungus (Trichomycetes), Amoebidium parasiticum. The fungi were present on the appendages singly or in bunches (see Taylor, 1928, Green, 1974). The close similarity of the feeding rate curves between Experiments 4 and 5 would indicate that the epibiont did not affect the feeding rate.

During the course of this experiment, some clumping of the algal cells was seen in the preserved subsamples, primarily in the Daphnia jars. The clumping appeared to increase

Table 15. Experiment 5. 270/1. Initial average cell concentrations (C_o) and subsampling intervals in hours. 5/81.

Jar	C_o	Subsampling intervals
1a	81165	0, 2, 4, 6, 8, 10, 12
1b	80613	"
2a	127963	"
2b	124121	"
3a	197392	"
3b	201230	"

Table 16. Clumps of C. reinhardi/ml over time in subsamples from Experiment 5. c jars are controls, no Daphnia.

	Interval time, hours			
Jar	0	4	8	12
1a	60	121	20	40
1b	60	60	50	20
1c	0	181	151	60
3a	121	241	542	1477
3b	151	121	573	753
3c	211	181	90	60

with time, particularly in jar 3 which had the highest algal level. Counts were made of the clumps and are presented in Table 16. Clumping was less frequent at lower algae levels, which would rule out the absence of NH_4Cl as a major factor in the clumping. Perhaps this phenomenon is related somehow to the repressed feeding rates seen in the high density Daphnia cultures at higher cell levels (see analysis, Ch.8). The clumping itself involves only a minor fraction of the cells. Most clumps were composed of 4-6 cells each. At the end of 12 hours, jars 3a and 3b still had 88,071 and 71,195 cells/ml respectively. This clumping was extremely rare during all of the experiments.

The calculated feeding rates and interval ln averaged C_A values are given in Table 17. These data also indicate a repression in feeding rates in crowded Daphnia as was observed in Experiment 4. The curves are remarkably similar. The low density Daphnia (30/liter) feed at rates considerably higher than the maximum rate of around 38,000 cells $D^{-1}h^{-1}$ at the higher cell concentrations tested. The five feeding rate curves generated as an Ivlev plot (see Ch.8) are included here in Figures 7, 8, 9, 10 and 11. The curve for Daphnia at a density of 90/liter falls between those of 30 and 270/liter. These data demonstrate a marked density dependence in the feeding rate of D. pulex, and will be analyzed and discussed following the chapter on the mathematics of feeding rates.

Table 17. Experiment 5. 270/1. Calculated feeding rates (f) and interval cell concentrations (C).

Jar	Hrs Sampling Interval	C	f
1a	0 - 2	71184	34692
1b	0 - 2	72074	29950
2a	0 - 2	110010	61828
2b	0 - 2	113145	38856
3a	0 - 4	181615	28043
3b	0 - 4	173249	38681
1a	2 - 4	49834	41948
1b	2 - 4	57182	25369
2a	2 - 4	88491	21811
2b	2 - 4	93974	32437
1a	4 - 6	35752	14161
1b	4 - 6	42657	27556
2a	4 - 6	72494	35787
2b	4 - 6	71815	47017
1a	6 - 8	24403	25183
1b	6 - 8	27651	26926
2a	6 - 8	53854	32926
2b	6 - 8	54810	19185
3a	4 - 8	143404	40774
3b	4 - 8	130344	39831
1a	8 - 10	13864	14928
1b	8 - 10	16329	16324
2a	8 - 10	38519	22485
2b	8 - 10	38501	37320
3a	8 - 12	104108	32403
3b	8 - 12	88736	36484
1a	10- 12	8451.1	6455.6
1b	10- 12	9706.6	9207.6
2a	10- 12	26742	19402
2b	10- 12	24573	17417

Figure 7. Experiment 1, Ivlev plot of C_A and f values. C_A here is the arithmetic average of the cell concentration.

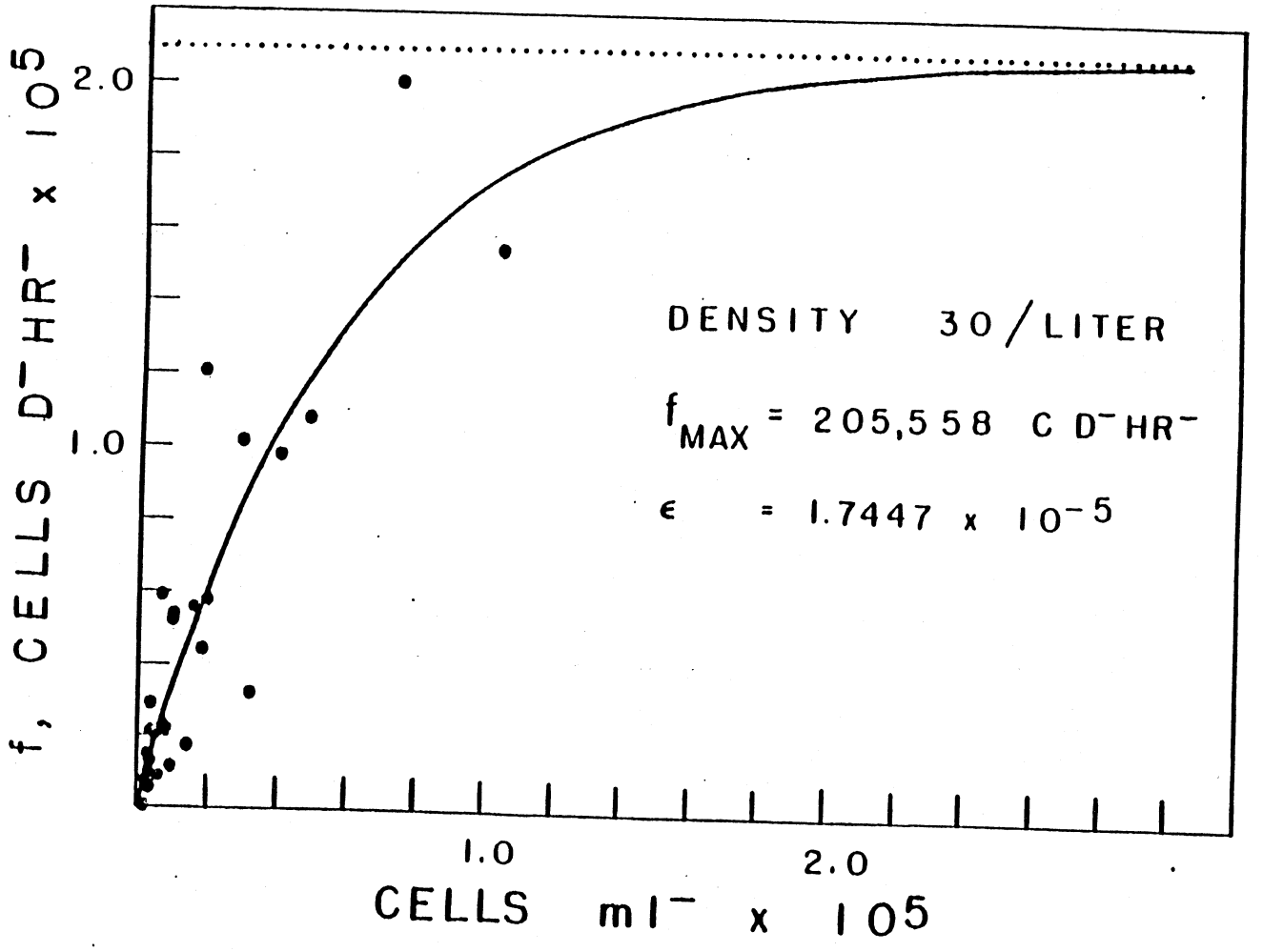

f, CELLS D⁻HR⁻ x 10⁵
2.0
1.0
DENSITY 30/LITER
f_{MAX} = 205,558 C D⁻HR⁻
ϵ = 1.7447 x 10⁻⁵
1.0
2.0
CELLS ml⁻ x 105

Figure 8. Experiment 2, Ivlev plot of C_A and f values. C_A here is the arithmetic average of the cell concentrations.

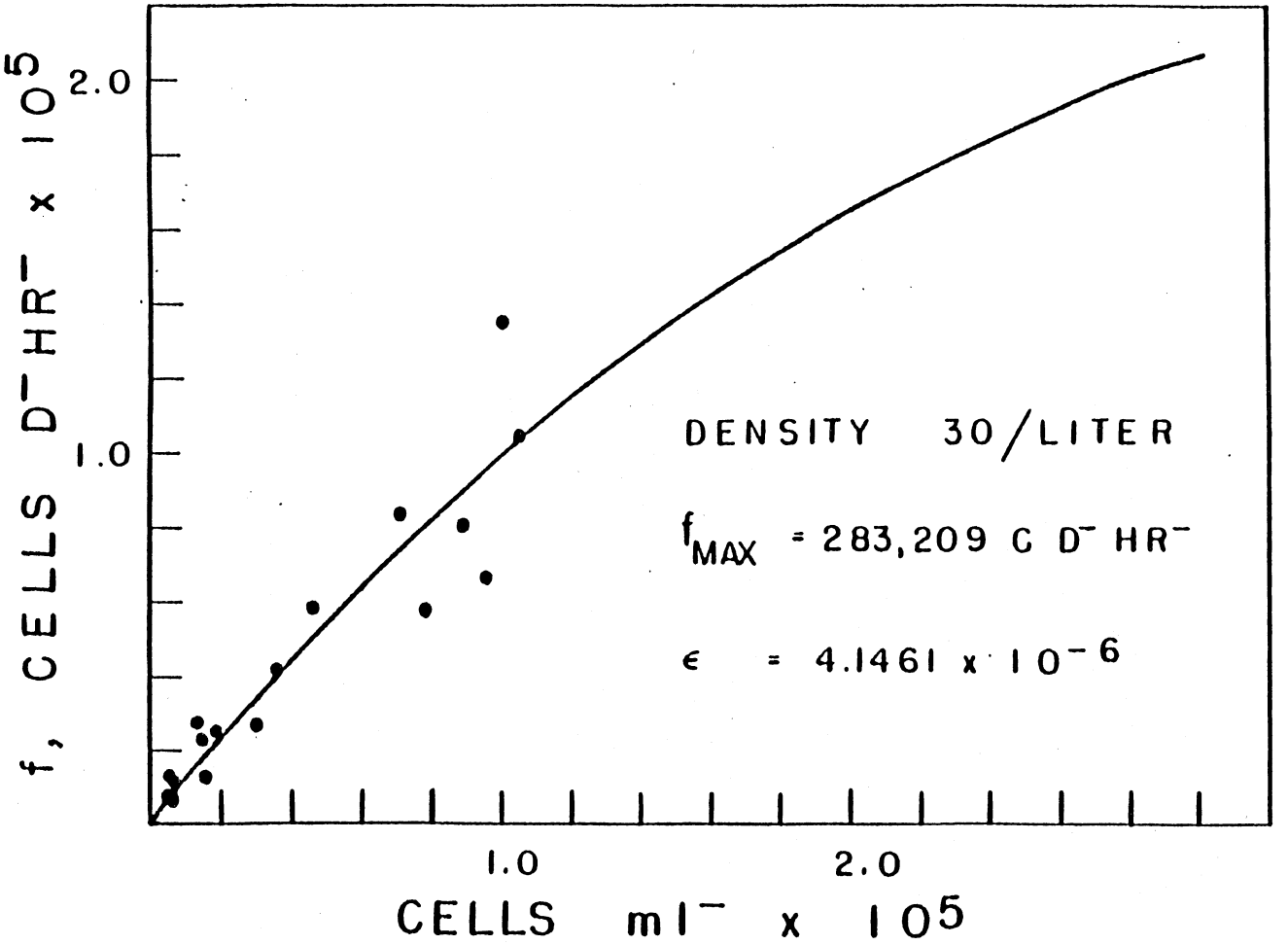

f, CELLS D⁻HR⁻ x 10⁵
2.0
1.0
DENSITY 30/LITER
f_{MAX} = 283,209 C D⁻ HR⁻
ϵ = 4.1461 x 10⁻⁶
1.0
2.0
CELLS ml⁻ x 10⁵

Figure 9. Experiment 3, Ivlev plot of C_A and f values. C_A here is the arithmetic average of the cell concentration.

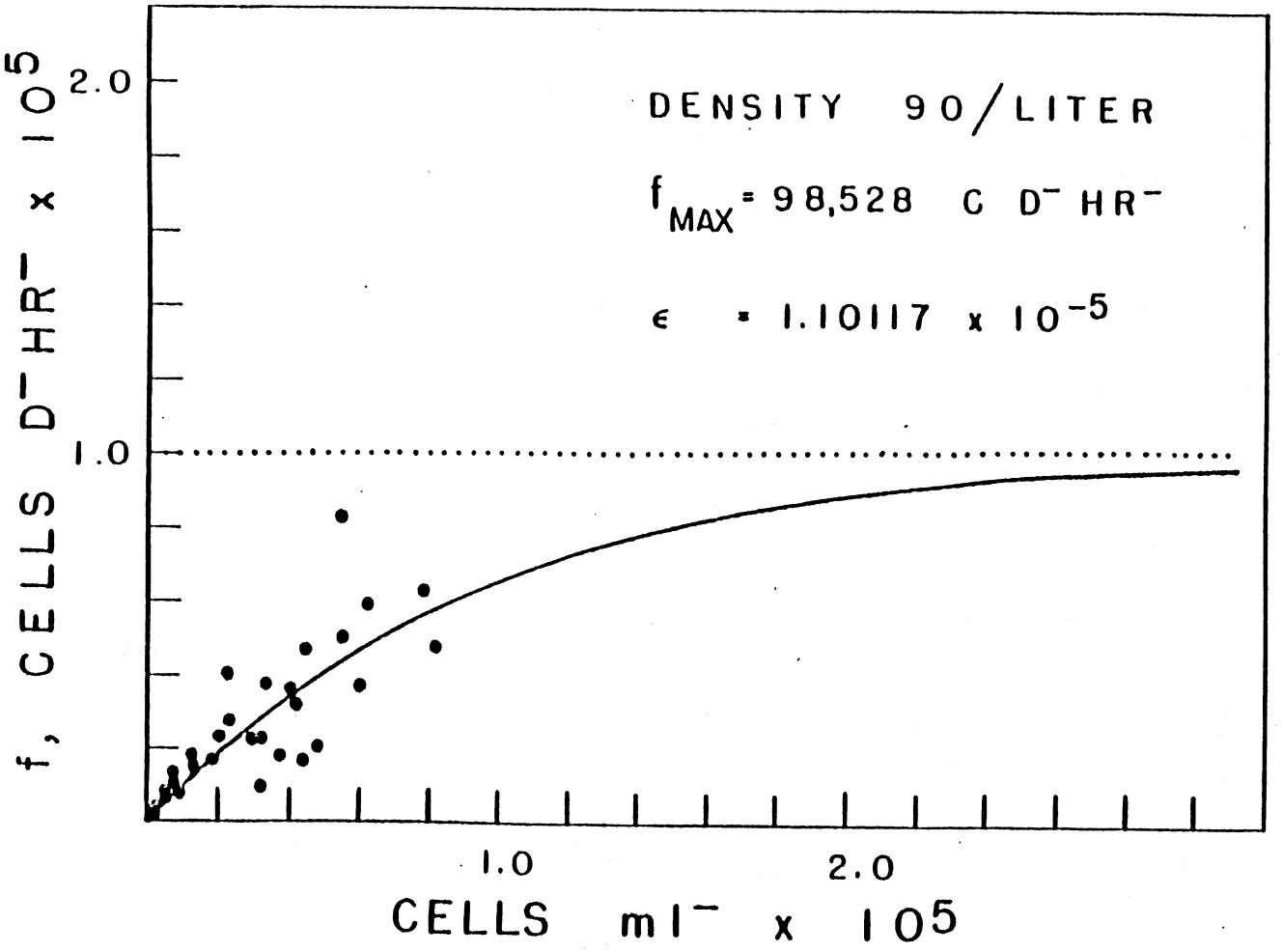

DENSITY 90/LITER
f_{MAX} = 98,528 C D⁻ HR⁻
ϵ = 1.10117 x 10⁻⁵
f, CELLS D⁻ HR⁻ x 10⁵
2.0
1.0
1.0
2.0
CELLS ml⁻ x 10⁵

Figure 10. Experiment 4, Ivlev plot of C_A and f values. C_A here is the arithmetic average of the cell concentrations.

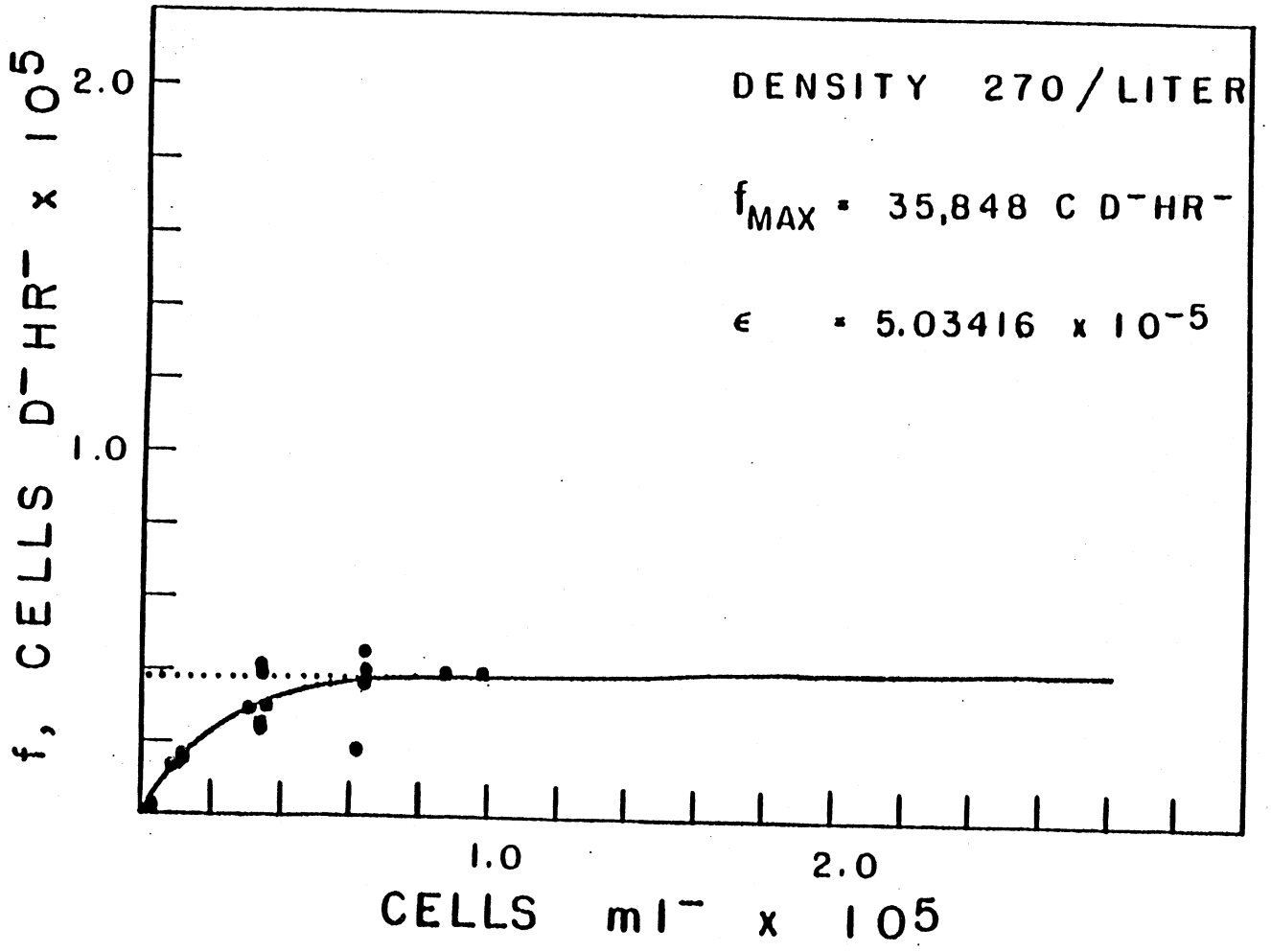
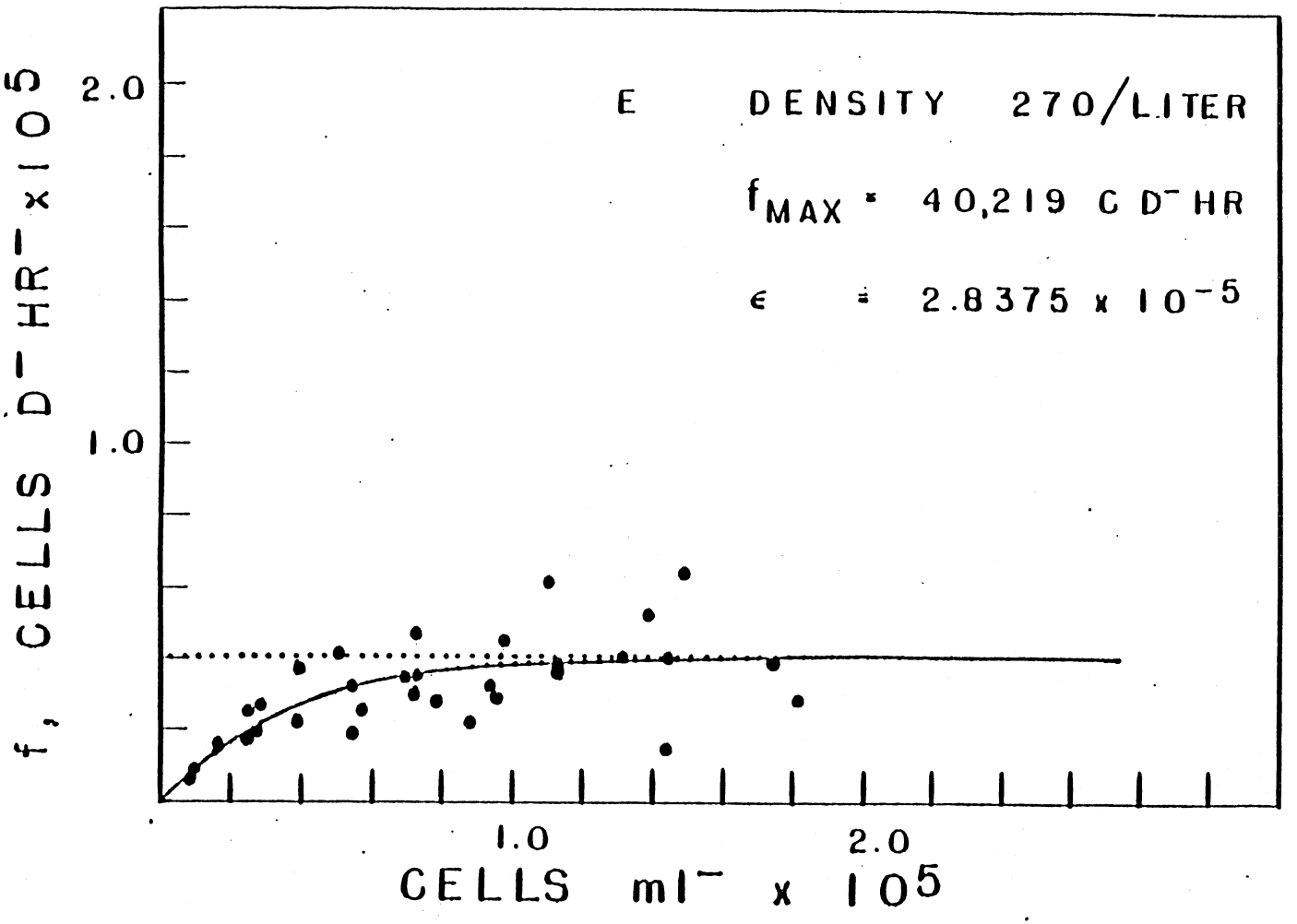

DENSITY 270/LITER
f_{MAX} = 35,848 C D$^-$HR$^-$
ϵ = 5.03416 x 10^{-5}
f, CELLS D$^-$HR$^-$ x 10^5
2.0
1.0
1.0
2.0
CELLS ml$^-$ x 105

Figure 11. Experiment 5, Ivlev plot of C_A and f values. C_A here is the arithmetic average of the cell concentrations.


E DENSITY 270/LITER
f_{MAX} = 40,219 C D⁻ HR
ϵ = 2.8375 x 10⁻⁵
f_1 CELLS D⁻ HR⁻ x 10⁵
2.0
1.0
1.0
2.0
CELLS ml⁻ x 10⁵

Chapter 7

Concepts and Mathematics of Feeding Rates

There are numerous ways to describe mathematically the data derived from analysis of feeding and filtering rates in zooplankton. Some are convenient because the parameters of the functions are easily derived, some fit a wide range of situations, but unfortunately only a few are conceptually satisfying. In fact, there may not be a universal function that will be satisfactory because some zooplankton show a clear saturation response in feeding (McMahon and Rigler, 1965; Frost, 1972) and others do not (Huntely, 1981), some show linearity in low food levels (Mayzaud and Poulet, 1978), and others have curvilinear rates. Furthermore, the feeding curve differs markedly for starved versus non-starved animals (Geller, 1975), and might be expected to differ for "edible" vs. "non-edible" algae. Lehman (1976), in his "energy optimization model," attempted to include the effects of differing gut passage times and digestion times.

Feeding rates can be treated conceptually as predation. The earliest suggestion that the rate of removal of prey by predators was a linear function of the prey density is to be found in the equations of Lotka (1923) and Volterra (1926). Their equations indicate that the change in the prey population, N_1, per unit time was partially a function of removal by the predators, N_2, having a constant attack coefficient "a." The predatory removal term $-aN_1N_2$ predicted that

consumption of prey would increase in a direct linear way as the prey density increased, with no upper limit to predation. One difficulty with the concept is the assumption of a constant attack coefficient "a," i.e., the slope of the predation curve. It is now abundantly clear that, in terms of feeding rates, "a" is actually declining regularly as N_1 (food) increases.

The assumption of a constant F or filtering rate for copepods means that constant filtering activity should cause an exponential decline in cell concentrations so the cell level at the end of a feeding interval would be

$$C_t = C_o e^{-kt}$$

with k as the slope of the linear transformation

$$\ln C_t = -kt + \ln C_o$$

and $F = vk$ where v = the volume per animal (Gauld, 1951). Using for the cell concentration the ln average for the interval, or

$$C_A = e^{\frac{(\ln C_o + \ln C_t)}{2}}$$

and assuming $f = F C$, then the feeding rate in Gauld's view would take the form

$$f = v \frac{\ln C_o - \ln C_t}{t} e^{\frac{(\ln C_o + \ln C_t)}{2}}$$

Retention of the basic linear model with inclusion of a reduction of f at higher food levels can be described by a rectilinear model where two regression functions are derived over the range of food levels, and the "breaking point" or x is given (e.g., Rigler, 1961). There are then, two constant F rates, the two slopes. This model would be easiest to derive for saturation type feeding, and most difficult in the curvilinear model. The desire to fit feeding rate data to straight lines has cause some workers to draw a line through one data point (cf Geller, 1975, Figs. 11, 15) or through a few points (cf McMahon and Rigler, 1965, Fig.1).

Curvilinear models are preferred for zooplankton feeding rates, particularly for rates of feeding populations. A power function of the form $f = k C^a$ would reduce to a linear form as "a" approaches 1, but is not suitable for saturation data. Holling's "basic functional response equation" (Holling, 1959) describes "Type II" predation as a negatively accelerating rise to a plateau

$$\frac{N_a}{N_t} = \frac{a N_o}{1 + aT_h N_o}$$

where N_a = number of prey eaten

T_t = time interval

N_o = prey density

a = a constant, the "instantaneous discovery rate" (area swept x ratio of successful captures to total contacts)

T_h = handling time, time to pick up a disc, or to search, kill, digest

The coefficients "a" and T_h can be derived from the linear transformation where

$$y = N_a T_t^{-1}$$

$$x = N_o \quad \text{and} \quad T_t = 1 \text{ hr},$$

then $\frac{y}{x} = -a T_h y + a$ with the slope = aT_h and the intercept = a. The quantity $\frac{y}{x}$ is analogous to F, the filtering rate, and x or N_o analogous to C the cell concentration, so the transformation would require plotting F or $\frac{(\text{number attacked/hr})}{N_o}$ as the dependent variable on the y axis against f as x, making the feeding rate the independent variable. Inclusion of f on both sides will make the estimates more subject to error because there will be more error in f measurements than in C values.

The Holling equation is not useful because it does not fit conceptually with zooplankton feeding, nor does it include within it the saturating feeding rate. A rearrangement of the Holling equation claimed by Williams (1980) to be analogous to the Michaelis-Menten function is:

$$\frac{N_a}{N_t} = \frac{(\frac{1}{T_h})\ N_o}{\frac{1}{aT_h} + N_o} = \frac{F_2\ M_1}{K_2 + M_1}$$

where F_2 is maximum predation, M_1 is the prey density, and K_2 the half saturation constant. This makes $\frac{1}{T_h}$ in units of reciprocal hours analogous to a maximum predation rate in units of prey eaten predator$^{-1}h^{-1}$, "when all available time is occupied in 'handling'."

The reciprocal of the handling time $\frac{1}{T_h}$ can be analogous to maximum feeding rate, f_{max}, if one assumes that Rashevsky's concept for fish is correct: that there is a finite time r to process each food particle, i.e., the fish can process only one particle every r seconds, and that in a heavy concentration of particles, there will be a maximum number that can be ingested defined by

$$\frac{\text{feeding interval}}{\text{time to eat each particle}} = \frac{1}{r}$$

(Rashevsky, 1959). The limitation imposed by processing time means that there is a maximum feeding rate. This concept may have validity.

No obvious analogy can be made between $\frac{1}{aT_h}$ and the half saturation constant K_2, which would be the concentration

of prey at which the predator eats at half the maximum rate. It is difficult to translate units of reciprocal area x time into prey density.

The Monod function or Michaelis-Menten equation is a commonly used rectangular hyperbola in which the maximum specific growth rate (μ_m) or the enzyme velocity (V_{max}) is included where C, a nutrient level, or S, the substrate concentration are rate limiting. K_μ and K_m are the concentrations at which half maximum velocities are reached. It is necessary that the particular nutrient or substrate be limiting, all others in excess. For enzymes, the natural concentrations of substrates are usually well below the K_m, this may not be true for zooplankton.

$$= \frac{\mu_{max}\ C}{K_\mu + C} \qquad v = \frac{V_{max}\ S}{K_m + S}$$

Monod Michaelis-Menten

While the equation does not describe the slope of the rise to saturation, the initial rate of reaction can be defined by "tangent α ", or $\frac{V_m}{K_m}$, giving the initial angle of increase at lowest S or C. In a slowly increasing curve, tangent would be under 2, the angle around 60° and in a rapidly rising curve, tangent α could be around 10, the angle over 84°.

The parameters of the Michaelis-Menten function are commonly estimated by use of the linear transformations below:

1. $\frac{C}{f} = \frac{K_m}{f_m} + \frac{1}{f_m}(C)$ $\quad y = \frac{C}{f} \quad x = C$

2. $f = f_m - K_m\left(\frac{f}{C}\right)$ $\quad y = f \quad x = \frac{f}{C}$

3. $\frac{1}{f} = \frac{1}{f_{max}} + \left(\frac{K_m}{f_m}\right)\left(\frac{1}{C}\right)$ $\quad y = \frac{1}{f} \quad x = \frac{1}{C}$

Depending on the type of error in the data, these transformations are subject to large errors in the estimates. The frequently used double reciprocal plot, equation 3, is the most susceptible to error (Down and Riggs, 1965). The parameters can also be estimated graphically, (Eisenthal and Cornish-Bowden, 1974), but the procedure involves drawing a line for each data pair and listing the intersections to find the median value, a tedious procedure with a large data set unless done on a computer.

In order for the least squares method for estimation of the error around the parameters to have validity (Cleland, 1967), the error around the estimation of S or C concentrations must be very small, while the error around the velocities would be larger.

The use of the Michaelis-Menten function to describe predation rates has been criticized because of the assumption

of the steady state (Williams, 1972, 1980), which for enzyme kinetics means that the rate of formation of the ES complex is equal to the rate of breakdown; the K_m equals the sum of the rate constants for breakdown divided by that of formation:

$$E + S \underset{k_2}{\overset{k_1}{\rightleftharpoons}} ES \xrightarrow{k_3} E + P$$

$$K_m = \frac{k_2 + k_3}{k_1}$$

These rate constants could be conceptualized in terms of zooplankton feeding activities as follows:

k_1 = filling rate of food groove, or collection rate, affected by filter appendage rate and carapace gape

k_2 = probably small, could be rejection rate by postabdominal claw when food is in excess

k_3 = rate of emptying of food groove, including swallowing frequency, size of bolus, mandibu- rate, peristaltic rate

The analogy to the saturatable enzyme E would be the holding capacity of the food groove assuming no postabdominal rejection. Like the enzyme its "capacity" is regenerated. At saturation ES is analogous to a full food groove. This could be measured by having food in excess, then decreasing it until the point at which postabdominal rejection stops

(or changes markedly), assuming postabdominal rejection is ridding the groove of excess food. Below saturation, k_i, or the filling rate, is limiting, and at the steady state

$$\text{concentration in food groove} = \frac{(\text{food groove capacity})\ C}{K_m + C}$$

and

$$K_m = \frac{(\text{PA rejection rate}) + (\text{FG emptying rate})}{\text{collection rate}}$$

where C is the food level, PA is the postabdomen, and FG the food groove.

Perhaps the most useful function to describe feeding rates is the widely used Ivlev or Gause equations. Used by Gause (1934) for predator increase at various levels of prey, the equation took the form

$$\frac{1}{N_2}\frac{dN_2}{dt} = b_2 (1 - e^{-N_1})$$

where

N_1 = prey density

N_2 = predator density

b_2 = the maximum rate of increase of N_2

Gause assumed that the rate of increase was proportional to "still unutilized opportunity for increase," or

$$\frac{dy}{dx} = k\,(a - y)$$

as the slope of the function, and integrated as above

$$y = a\,(1 - e^{-kx})$$

with a = the maximum rate of predator increase.

Ivlev used this function to describe feeding rates in various fish such as carp, roach and bleak in the form

$$r = R\,(1 - e^{-\epsilon p})$$

where r was the ration eaten, and R the maximum ration. ϵ was the coefficient of proportionality, and p the concentration of food supplied. Ivlev's (1961) feeding rate work has been criticized because the fish were starved 18-20 hours prior to an experiment so that gut analysis could be made. Despite this objection, the function itself is useful because it contains the saturating value R as well as a coefficient ϵ that describes the rate of increase to R as food levels increase, that is, the derivative is

$$\epsilon\,(R - r)$$

In the calculations of feeding rates in this study, the Ivlev or Gause equation will have the form

$$f = f_{max} (1 - e^{-\varepsilon x})$$

$$\frac{df}{dx} = \varepsilon (f_m - f)$$

where f_{max} or f_m = maximum feeding rate and x = food level. The coefficient ε describes the rate of increase to f_{max}. A large value of ε, around 5×10^{-5}, describes a rapid increase to f_{max}; a low ε of 5×10^{-6} means a very slow increase of feeding rate with food level. The effects of varying ε and f_{max} on the Ivlev function are shown in Figures 12 and 13. Feeding rate curves with a large ε value show definite saturation, those with low ε could possible be described with a linear function.

The parameters f_{max} and ε can be estimated by iteration either by using a linear transformation for which there is only one f_{max} giving a straight line, or by a minimum likelihood estimate using a Taylor's series (Marquardt, 1063). A linear transformation that can be used is

$$\ln (f_{max} - f) = \ln f_{max} - \varepsilon x$$

Values of f_{max} are inserted into the equation, and only one value will result in a straight line whose slope depends on ε.

The Ivlev function was converted to a hyperbolic function by Rashevsky (1959), who interpreted fish feeding rates

Figure 12. The effect of varying the coefficient ε on the shape of the Ivlev function curves, with f_{max} held constant.

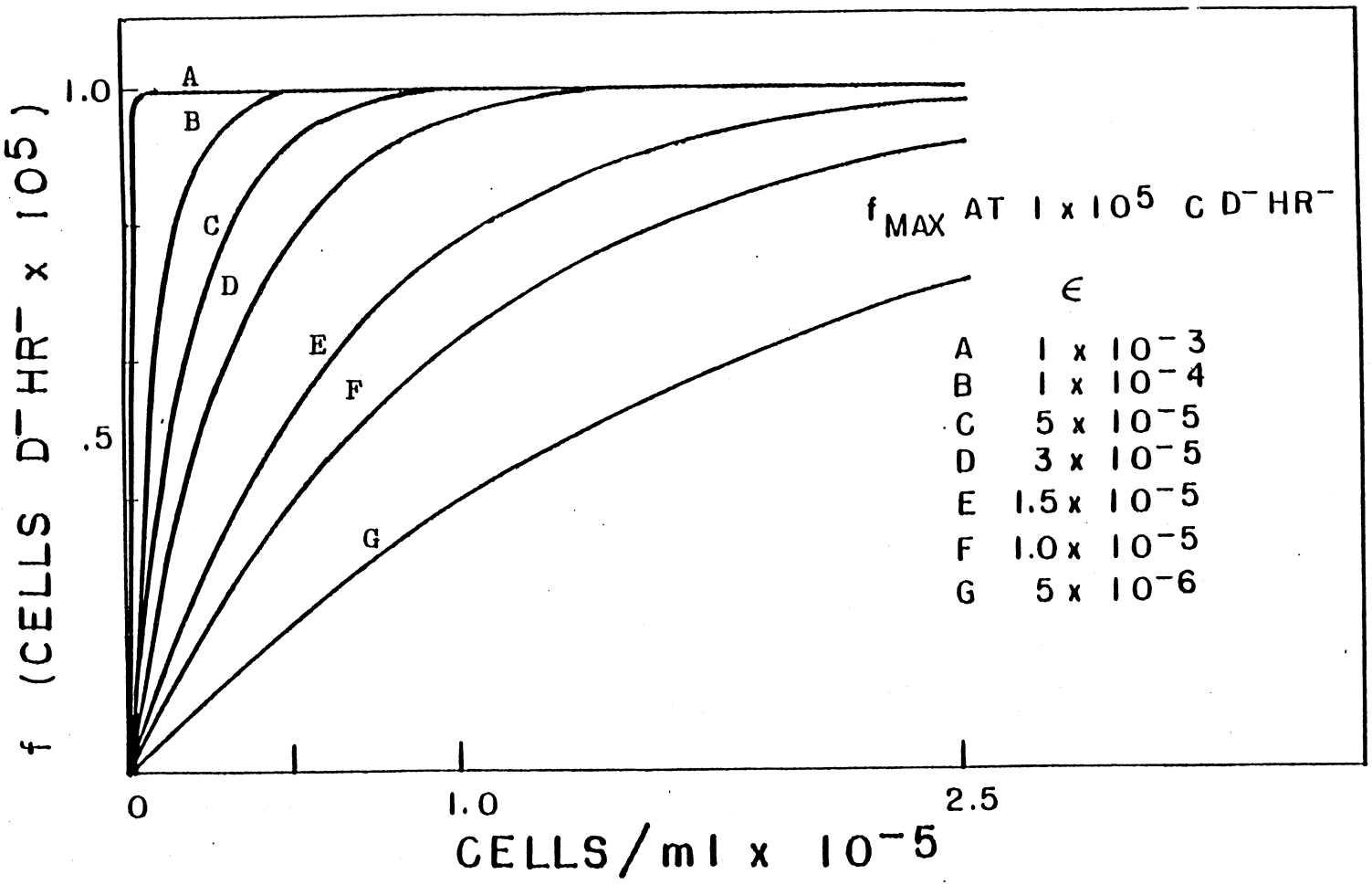
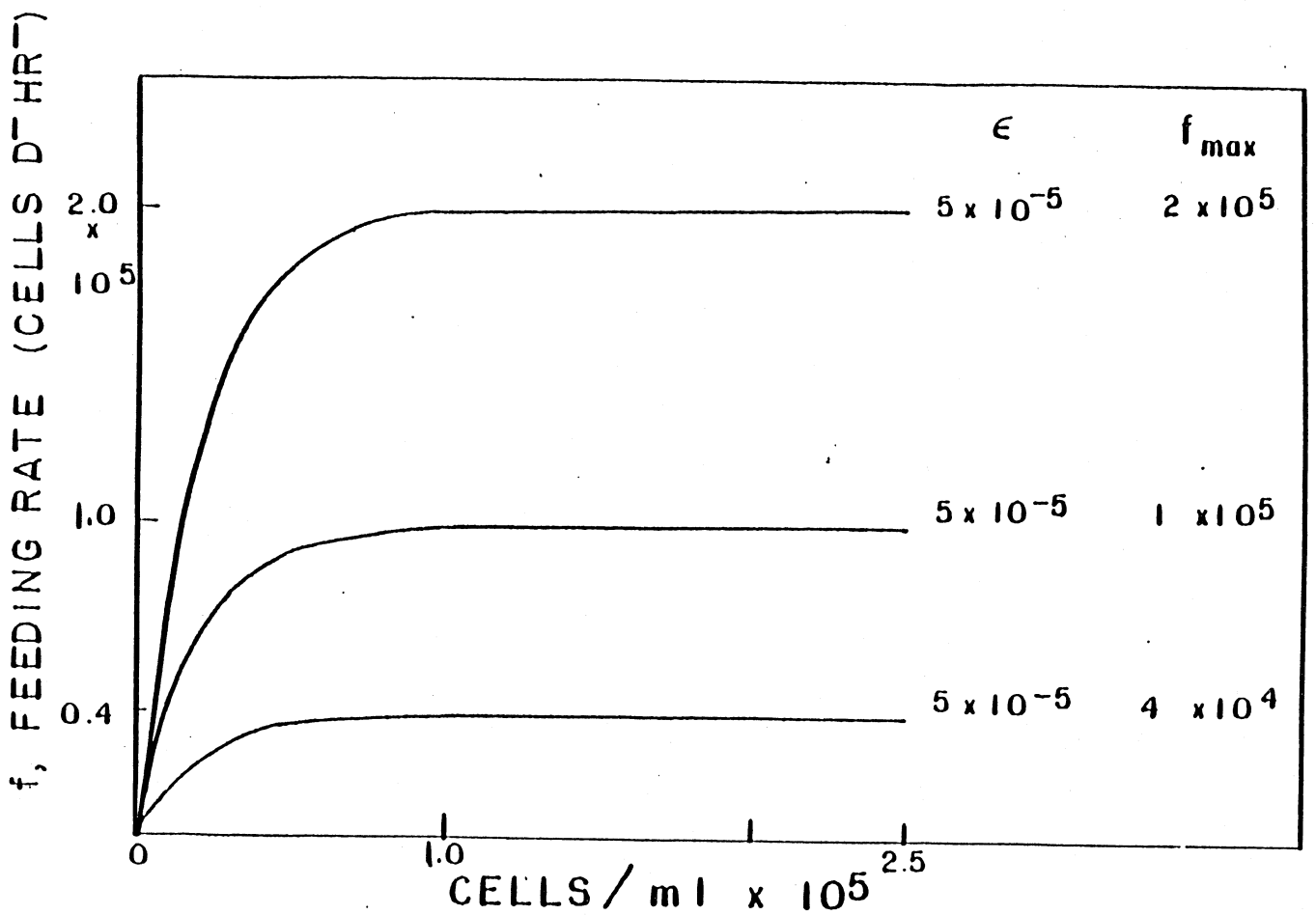

f (CELLS D⁻HR⁻ x 10⁵)
1.0
.5
0
1.0
2.5
CELLS/ml x 10⁻⁵
A
B
C
D
E
F
G
f MAX AT 1 x 10⁵ C D⁻HR⁻
ϵ
A 1 x 10⁻³
B 1 x 10⁻⁴
C 5 x 10⁻⁵
D 3 x 10⁻⁵
E 1.5 x 10⁻⁵
F 1.0 x 10⁻⁵
G 5 x 10⁻⁶

Figure 13. The effect of varying f_{max} on the shape of curves from the Ivlev function, coefficient ε is held constant.


f, FEEDING RATE (CELLS D⁻ HR⁻)
2.0 x 10⁵
1.0
0.4
0
1.0
2.5
CELLS / ml x 10⁵
ε
f max
5 x 10⁻⁵
2 x 10⁵
5 x 10⁻⁵
1 x 10⁵
5 x 10⁻⁵
4 x 10⁴

in terms of gut filling rates. At steady state

$$\frac{dm_i}{dt} = 0$$

and ingestion = a loss function, km_i, where m_i is the proportion of gut fullness. Assuming that gut filling approached asymptotically the value

$$m_i = \frac{x}{\frac{x}{M} + k} \qquad (M = \text{maximum fullness})$$

and substituting this for k, he arrived at

$$\text{Ingestion, stationary} = \frac{x\ k\ M}{x + kM}$$

which is a rectangular hyperbola that requires direct measurement of k, the constant of "absorption" from the gut. This function is not useful for zooplankton feeding rates because the loss function should include assimilation, excretory and respiratory losses of ingested food to be complete. All of these would be expected to feed back in a regulatory way to the feeding rate. Also, it is important to realize the assimilation rates are not a simple linear function of gut fullness. Assimilation rates at various food levels show curvilinear or rectilinear curves fitted by rectilinear, Ivlev, or Michaelis-Menten functions (Lampert, 1977).

Chapter 8

Analysis of Feeding Rate Data From Experiments 1 - 5

The five data sets from the feeding rate experiments have been analyzed using the Michaelis-Menten and the Ivlev functions. The parameters f_{max} and K_m for the Michaelis-Menten function can be derived, as discussed before, by three linear transformations of the equation given in the preceding section. These three require, in the order given, plotting or regressing C (as y) vs $\frac{C}{f}$, f (as y) vs $\frac{f}{C}$, or $\frac{1}{f}$ (as y) vs $\frac{1}{C}$, the well known double reciprocal plot. Each transformation was performed on the five data sets using a program for linear regression/ANOVA on the Hewlitt Packard calculator with plotting capability. The results are shown in Tables 18, 19, 20, 21, and 22. Included are the estimates of f_{max} and K_m as well as β, the slope of the regression, r, the correlation coefficient, the ANOVA F ratio, the appropriate value of F for the .05 level from tables, the result of testing the hypotheses H_o : $\beta = 0$, and the 95% confidence interval around the slope β. The transformation of f vs $\frac{f}{C}$ gives very low correlations with the data sets, and the F values for this transformation in four of the five data sets are so low that the hypothesis that the slope is zero is accepted.

Correlations for the transformation where C is plotted against $\frac{C}{f}$ are low for three sets of data, and high only for

Table 19. Experiment 1. 30/liter. Analysis of Michaelis-Menten linear transformations of the data given in Table 5.

Transform	$\frac{C}{f}$ vs C	f vs $\frac{f}{C}$	$\frac{1}{f}$ vs $\frac{1}{C}$
f_{max}	224901	75707	89031
K_m	157000	23937	48786
β	4.445×10^{-6}	-23937	.54787
r	.516	-.336	.851
F	5.445	1.907	3.949
$F_{.05}$	6.20	6.20	6.20
$H_o: \beta = 0$	Acc	Acc	Acc
95% CI, β	$\pm 4.06 \times 10^{-6}$	$\pm$ 36938	$\pm$ .1859

Table 20. Experiment 2. 30/1. Analysis of Michaelis Menten linear transformations of the data given in Table 8.

Transform	$\frac{C}{f}$ vs. C	f vs. $\frac{f}{C}$	$\frac{1}{f}$ vs. $\frac{1}{C}$
f_{max}	-101615	34508	-7171
K_M	-52730	3434	-11461
β	-9.841×10^{-7}	3434	1.598
r	-.03049	.1685	.5753
F	.02512	.7889	13.354
$F_{.05}$	5.63	5.63	5.63
$H_o: \beta = 0$	Acc	Acc	Rej
95% CI, B	$\pm 1.274 \times 10^{-5}$	$\pm$ 7934.6	$\pm$ .8975

Table 21. Experiment 3. 90/1. Analysis of Michaelis Menten linear transformations of the data given in Table 11.

Transform	$\frac{C}{f}$ vs. C	f vs. $\frac{f}{C}$	$\frac{1}{f}$ vs. $\frac{1}{C}$
f_{max}	68278	34266	66408
K_M	48230	9820	41937
β	1.465×10^{-5}	-9820	.6315
r	.4972	-.2519	.9813
F	11.16	2.3035	182.89
$F_{.05}$	10.9 (.005)	5.57	5.57
H_o: $\beta = 0$	Rej	Acc	Rej
95% CI, β	$\pm 8.907 \times 10^{-6}$	$\pm$ 13147	$\pm$ .0949

Table 22. Experiment 4. 270/1. 3/81. Analysis of Michaelis Menten linear transformations of the data given in Table 14.

Transform	$\frac{C}{f}$ vs. C	f vs. $\frac{f}{C}$	$\frac{1}{f}$ vs. $\frac{1}{C}$
f_{max}	43548	38213	189226
K_M	21728	13486	151157
β	2.2963×10^{-5}	-13486	.79882
r	.8257	-.5357	.9905
F	34.27	6.439	827.3
$F_{.05}$	6.12	6.12	6.12
$H_o: B = 0$	Rej (p .001)	Rej	Rej (p .001)
95% CI, β	$\pm 8.315 \times 10^{-6}$	$\pm$ 11166	$\pm$ .0584

Table 23. Experiment 5. 270/1. 5/81. Analysis of Michaelis Menten linear transformations of the data given in Table 17.

Transform	$\frac{C}{f}$ vs. C	f vs. $\frac{f}{C}$	$\frac{1}{f}$ vs. $\frac{1}{C}$
f_{max}	42976	37177	56980
K_M	27887	13860	53166
β	2.3269×10^{-5}	-13860	.93306
r	.8704	-.3149	.912
F	87.54	3.083	138.5
$F_{.05}$	5.61	5.61	5.61
$H_o: \beta = 0$	Rej	Acc	Rej
95% CI, β	$\pm 1.271 \times 10^{-5}$	$\pm$ 16166	$\pm$ .1619

the data of sets 4 and 5. The F ratios for both 30/liter sets indicate acceptance of a zero slope. The double reciprocal transformation appears outstanding on the basis of four of the five r values, but the estimates of f_{max} are weird for experiments 2 and 4. Dowd and Riggs (1965) predicted that the most robust transformation was f (as Y) against $\frac{f}{C}$, especially when the error around f was large and either constant or variable. However, with the data here, the r values in Experiments 2 and 3 are low for this transformation, as are the estimates of f_{max}.

None of the transformations of the Michaelis-Menten equation fit the present data. This function is not the best equation for describing feeding rates in Daphnia at various densities.

The Ivlev function

Originally the parameters of the Ivlev function, f_{max} and ε were estimated by trial and error by inserting various f_{max} values into the linear transformation below:

$$\ln (f_{max} - f) = \ln f_{max} - \varepsilon x$$

Only the true value of f_{max} results in a straight line with slope of ε. An iterative program written by Gordon Plorin proved invaluable in rapidly estimating the parameters ε and f_{max} by a minimum likelihood estimation procedure using

a Taylor's series and gave estimates of the variances around the parameters. The estimates of the variances and of the parameters by this method are presented in Table 24 and the resulting Ivlev functions are presented in Table 25.

It can be seen that the variances around these estimates are very small. The Ivlev function is preferred for describing these feeding rates not only because it fits the data very well, but also because it really is more descriptive than the Monod type function, because ε describes the rate of increase to f_{max}. It describes very well the full range of curves from the very saturating 270 Daphnia/liter curves to those at 30 Daphnia/liter.

A value analogous to the K_m of the Michaelis-Menten function, that is, the concentration C at which half the maximum feeding rate is achieved (K_c) can be derived from the Ivlev function as follows:

$$\text{call } \tfrac{1}{2} f_{max} = f_{.5m}$$

then, for the K_c cell concentration

$$f_{.5m} = f_m (1 - e^{-\varepsilon x})$$

$$\frac{1}{2} = (1 - e^{\varepsilon x})$$

so

$$e^{-\varepsilon x} = \frac{1}{2}$$

or

$$x = \frac{\ln 2}{\varepsilon} = K_c$$

These values have been calculated are are in Table 26.

The density dependent Ivlev feeding function

An Ivlev equation for density dependent feeding rates in Daphnia can be derived that includes the variable of Daphnia density, P, as well as the variable for food level C. Regressions of densities P as x with ε as y give the following results:

1. ln ln regression (power function)

   Slope = .6283 = β

   Intercept = -13.693

   b = 8.455×10^{-6}

   r = .768

2. semi ln regression, x vs. ln y (exponential function)

   Slope = .005905 = β

   Intercept = -11.681

   r = .807

The second regression is chosen because it provides a slightly better fit. In its linear form this is

$$\ln \varepsilon = \ln b = a P$$

and the exponential form is

Table 24. Parameters and variances of the Ivlev function obtained by iteration of data from Expts. 1-5 in Tables 5,8,11,14 and 17.

Expt.	Density	f_{max}	f_m s^2	λ	λ s^2
1	30/1	227031	207.7	5.5207×10^{-6}	1.9626×10^{-19}
2	30/1	197030	2.9170	2.0608×10^{-5}	8.1474×10^{-20}
3	90/1	90787	19.447.	1.2418×10^{-5}	6.7894×10^{-19}
4	270/1	35123	.2165	5.9545×10^{-5}	7.1716×10^{-18}
5	270/1	38841	.15484	3.0218×10^{-5}	8.2890×10^{-19}

Table 25. Ivlev equations for Daphnia feeding rates for data from Experiments 1-5 and different Daphnia densities. x = cell level.

Expt.	Density	Ivlev function
1	30/1	$f = 227031 (1 - e^{-5.5207 \times 10^{-6} x})$
2	30/1	$f = 197030 (1 - e^{-2.0680 \times 10^{-5} x})$
3	90/1	$f = 90787 (1 - e^{-1.2418 \times 10^{-5} x})$
4	270/1	$f = 35123 (1 - e^{-5.9545 \times 10^{-5} x})$
5	270/1	$f = 38841 (1 - e^{-3.0218 \times 10^{-5} x})$

Table 26. Half saturation constants, K_c, derived from the Ivlev functions for Experiments 1-5.

Experiment	Density	.5 f_{max}	$\epsilon \times 10^{-5}$	K_c
1	30/1	113516	.55207	125554
2	30/1	98515	2.0608	33636
3	90/1	45394	1.2418	55818
4	270/1	17562	5.9545	11641
5	270/1	19421	3.0218	22938

$$\varepsilon = b\, e^{aP}$$

or $$\varepsilon = (8.455 \times 10^{-6})\, e^{.005905P}$$

which is the density function of the coefficient ε.

Regressions of the density (P as x) and f_{max} as y give the following results for the density dependence of f_{max} on P:

1. ln ln transformation of density P and f_{max}

   Slope = -.7042

   Intercept = 14. 969

   b = 3.168

   r = .997

2. semi ln transformation of density and ln f_{max}

   Slope = -.006946

   Intercept = 12. 353

   r = .976

Both fits are good, but Equation 1 is chosen for the density function of f_{max}, which is, in the linear form

$$\ln f_{max} = \beta \ln P + \ln k$$

and in the power form

$$f_{max} = k\, P^{\beta} \quad \text{or}$$

$$f_{max} = (3.168 \times 10^6)\ P^{-.794}$$

This equation describes the decline in maximum feeding rate with the increase in Daphnia density.

These density dependent functions of f_{max} and ε can then be reinserted into the Ivlev function, so that any feeding rate at any cell concentration and Daphnia density can be predicted.

$$f = f_{max}\ (1 - e^{-\varepsilon x})$$

the density dependent Ivlev function, then, is as follows:

$$f = (3.168 \times 10^6)\ P^{-.794}\ (1 - e^{-x(8.455 \times 10^{-6})e^{.00591}})$$

where x is the cell concentration and P the density of Daphnia.

For the densities used here, the values of f_{max} and ε calculated from the combined equation are presented in Table 27. These values fit the values derived from the data very well (Table 24). The equation predicts extremely high feeding rates for very low density Daphnia, at 1/liter, and very low rates at a density of 1000 Daphnia/liter. The equation could be improved with data from these densities, and should not be used outside the densities studied. Feeding rates predicted from the equation for the three densities studied

(Table 28 and Figure 14) show that the uncrowded Daphnia would be expected to feed at higher rates at even the very low cell concentrations. The curves in Figure 14 from the predictive equation compare very well with a combined plot of the actual curves shown in Figure 15.

Table 27. Calculated values of f_{max} and ϵ from the density dependent Ivlev feeding function for Daphnia.

P Density/liter	f_{max}	ϵ
30	212639	1.009×10^{-5}
90	88860	1.439×10^{-5}
270	37134	4.165×10^{-5}

Table 28. Some feeding rates predicted from the density dependent Ivlev feeding function for 3 densities of Daphnia.

C, cells/ml	Density/liter		
	30	90	270
1000	2136	1269	1515
5000	10465	6167	6980
10000	20415	11906	12649
30000	55554	31147	26488
50000	84270	45576	32505
100000	135143	67776	36557
200000	184396	83858	37125

Figure 14. Ivlev feeding curves predicted from the density dependent Ivlev function.

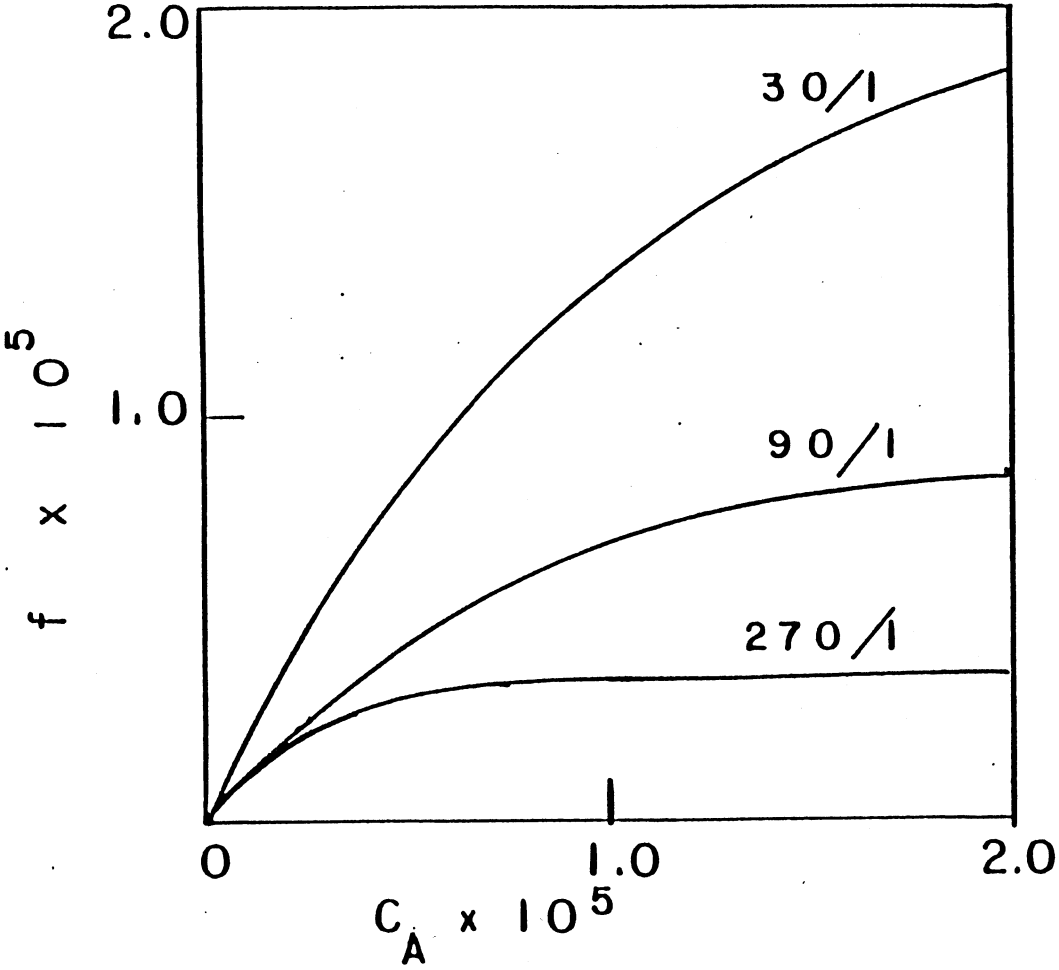
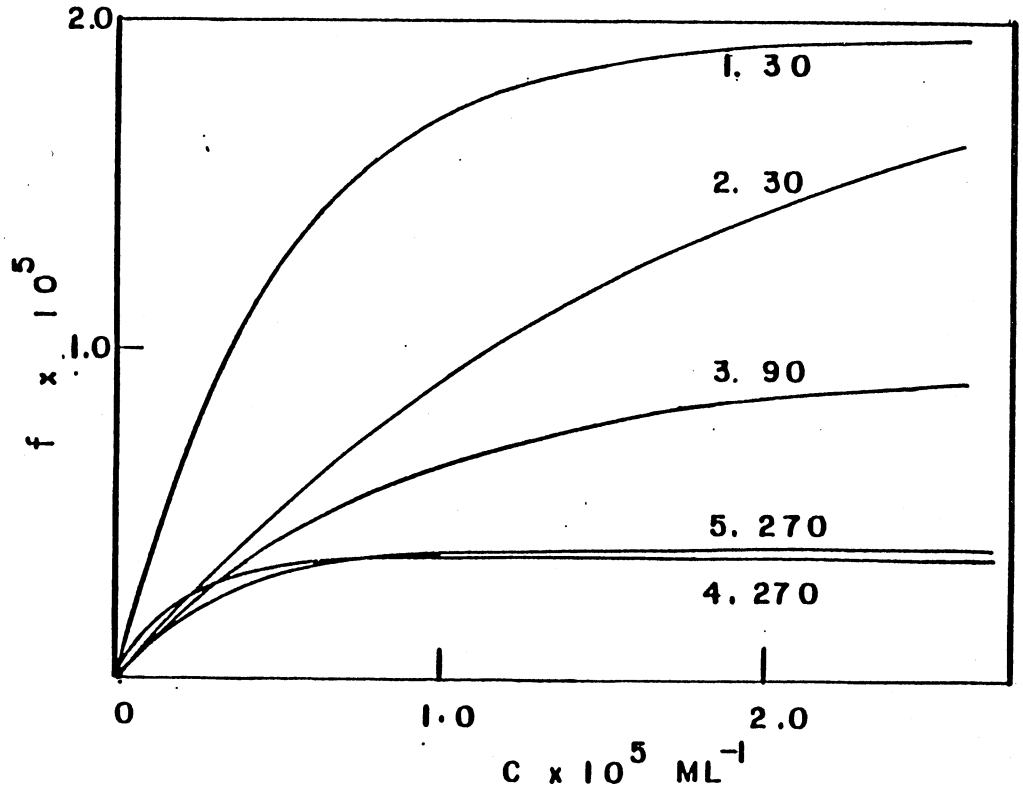

2.0
1.0
f x 10^5
30/1
90/1
270/1
0
1.0
2.0
C_A x 10^5

Figure 15. Ivlev feeding curves from Experiments 1 to 5, with C_A calculated as the ln average cell concentration.


2.0
1.0
f x 10^5
1. 30
2. 30
3. 90
5. 270
4. 270
0
1·0
2.0
C x 10^5 ML^-1

The equations derived from the density dependent Ivlev function, given in Table 29, compare very well with those obtained from the five data sets (see Table 25).

Table 29. Equations for feeding rates obtained from the density dependent Ivlev function for three densities of Daphnia.

Density			
30/1	f	=	$212639\ (1 - e^{-1.009 \times 10^{-5}x})$
90/1	f	=	$88860\ (1 - e^{-1.4386 \times 10^{-5}x})$
270/1	f	=	$37134\ (1 - e^{-4.165 \times 10^{-5}x})$

In conclusion, the work on feeding rates shows that the average ingestion rates of populations of Daphnia have a curvilinear increase as the cell concentration is increased. This means the decline in the filtering rate is also non-linear as the cell levels increase. For comparative feeding rate studies, it is important to report the volume as well as the numbers of the algal cells and any other measures of food levels such as carbon when working with a single species of algae. Filtering rates, when measured in the absence of food levels, are meaningless. Food levels should also be reported for feeding rates because of the dependency of f on C, unless the population exhibits a saturation feeding response and the

"incipient limiting level" is known.

Feeding rate measurements seem to be most reliable when they were carried out in particle free ½D64 medium with or without the vitamins and growth factors, and with added 0.005 g/liter NH_4Cl. The algae must be dark adapted overnight.

In Experiment 5, an increasing amount of clumping in C. reinhardi was seen in the presence of crowded Daphnia over 12 hours at the highest food level, but not in the control. It is tempting to speculate that the clumping is an adaptation in Chlamydomonas to avoid predation by Daphnia.

Analysis of the data from the five feeding rate experiments shows a poor fit to all three of the linear transforms of the Michaelis-Menten equation. On the other hand, the variances around the estimates of the Ivlev function are extremely small. The Ivlev equation is preferred for describing f rates, not only because of the close fit to the data, but also because it is more descriptive: the coefficient ϵ describes the rate of increase as the food level increases.

The results of the analysis of the feeding rates in the five experiments and three densities show clearly that the feeding rate in D. pulex is density dependent, with a marked depression at higher densities. The higher density feeding curve is clearly a saturating curve with the maximum feeding rate at approximately 37,000 cells $D^{-1}h^{-1}$. Lower density Daphnia feed at much higher rates and their f rate does not

saturate within the range of C tested. The meaning of the density dependence in feeding rate will be considered in Chapter 10.

A density dependent Ivlev feeding function for D. pulex has been derived, by inserting into the basic equation an exponential function for the density dependence of f_{max} and for the density dependence of ε. The final function back-predicts the observed data at the three densities of Daphnia very well.

Chapter 9

Allelopath Experiment

The mechanism for the repression of the feeding rate in crowded Daphnia pulex is not known. It is possible that crowded Daphnia, sensing the turbulence of other Daphnia, stop swimming and close the carapace in an avoidance reaction similar to the sinking reaction in chydorid cladocerans avoiding predators. To test this possibility, the degree of carapace gape and appendage movements could be measured photographically with single mounted Daphnia, using the method of Gliwicz (1980), in a chamber into which would be introduced other Daphnia to create different densities.

In Chapter 3 it was shown that reproduction of uncrowded Daphnia could be repressed in the presence of crowded Daphnia isolated behind a net barrier. In addition, there was some repression of growth, and the young born from uncrowded test females in this medium exhibited slow development and poor egg production when reared apart in fresh medium. When the crowded Daphnia were placed in dialysis tubing, reproduction in the uncrowded females was normal, indicating the "factor" was non-dialyzable. Recently, Folt and Goldman (1981) have demonstrated a repression of the filtering rate of the herbivorous copepod Diaptomus tyrrelli by Epischura nevadensis, which preys on the young of Diaptomus. The chemical "allelopath" released by Epischura into the water is non-dialyzable, and therefore of large molecular weight.

In the following experiment, the goal was to see if water preconditioned with crowded Daphnia would affect the feeding rate in uncrowded Daphnia. The ½D64 simplified medium was preconditioned with D. pulex at 300/liter, then the Daphnia and the algae used to feed the crowders were removed, followed by the addition of fresh dark-adapted C. reinhardi and the test Daphnia at 30/liter. As a control, feeding rates of Daphnia at 30/liter were measured in simplified ½D64 medium, called "RW" here, in the same way previous feeding rates have been determined.

The test medium, CCW, was preconditioned with crowded Daphnia for 12 hours before the test Daphnia were introduced. The uncrowded test Daphnia were preconditioned overnight in CCW at each cell level, and were transferred to new CCW in the morning for the incubation. There was no one hour preincubation. Other test Daphnia were incubated overnight in RW at each algal level and transferred in the morning to RW for the feeding incubation. The test procedure was complicated by the necessity to dark adapt the algae. First the CCW to be used had to be cleared of the crowded Daphnia and then filtered free of the algae on which they had been feeding. For the feeding incubation, dark adapted algae were collected by Millipore filtration and added to the cleared CCW medium before the uncrowded test Daphnia were introduced. In the controls, the test Daphnia were transferred to I jars in

in the morning from the overnight preincubation jars. Densities and initial cell concentrations are shown in Table 1. The uncrowded Daphnia (UC) were preconditioned at a density of 30/liter for three days before the experiment, fed 75000 cells/ml each day with daily water change. The Daphnia used to make the CCW or crowded conditioned water were kept crowded at a density around 300/liter for two days before they were used to make CCW, and fed 175,000 cells/ml at least once a day. The medium used for feeding incubations was particle-free as usual. At the time of harvest, C. reinhardi counts were 1.4×10^6 cells/ml or less.

Subsampling consisted of taking three 10 ml aliquots (samples a, b and c) from each jar at 0, 6 and 12 hours after the Daphnia were introduced, for a total of 54 samples. The initial t_o sample was taken 20 minutes after the Daphnia were added to allow time for the food groove to fill, and for the animals to overcome any disturbance from the transfer. In this way, the Daphnia are assumed to be eating in a normal way when the t_o subsample is taken for counts. The counts for the individual samples are given in Table 2. The means and standard deviations for the triplicate samples are in Table 3.

The usual preincubation time had to be eliminated because there was insufficient time to filter all the samples. Counts on the OVPI jars after the removal of the uncrowded

Table 1. Additions to experimental CCW medium and RW control medium for tests of feeding rates in Daphnia at 30/l.

Jar	Medium	Vol ml	# Daphnia	C_o	Notes
9	CCW	1500	45	54746	Algae dark-adapted in GD-64 overnight and then added to CCW at t_o by millipore filtration
10	CCW	1500	45	57245	
11	CCW	600	0	61577	
12	RW	1500	45	78641	Algae added to RW for overnight , used as usual for t_o in AM
13	RW	1500	45	80688	
14	RW	600	0	47840	

Table 4. Sizes of uncrowded Daphnia used to test feeding rates in CCW and RW media.

Jar	$\bar{X}$, mm	S.D.	Average for treatment
9	2.219	.1461	2.192
10	2.165	.1415	
12	2.208	.1154	2.203
13	2.198	.1152	

Table 5. Average cell counts of control algae in CCW (crowded conditioned) and RW (regular) media, no Daphnia.

Jar	Med	t_o	t_6	t_{12}
11	CCW	61577	48280	25025
14	RW	47840	56492	48807

Table 2. Cell counts for all samples in allelopath experiment. Cells/ml

Medium	Jar/sa	t_o	t_6	t_{12}
CCW	9a	57182	23317	14842
CCW	9b	51042	21660	14855
CCW	9c	56015	25126	18307
CCW	10a	58990	29947	20003
CCW	10b	59480	30588	17893
CCW	10c	53265	30023	19588
CCW-control	11a	58350	49950	25088
CCW "	11b	60686	50402	24448
CCW "	11c	65696	44488	25540
RW	12a	75678	34656	23129
RW	12b	79558	37067	17818
RW	12c	80688	35447	17968
RW	13a	81215	34581	21359
RW	13b	81969	36464	23694
RW	13c	78880	39553	27235
RW-control	14a	50025	53114	55449
RW- "	14b	50929	59179	48857
RW- "	14c	42567	57182	42114

Table 3. Average counts from Table 2.

Jar	t_o	S.D.	t_6	S.D.	t_{12}	S.D.
9	54746	2662	23368	1415	16035	1607
10	57245	2821	30186	286	19161	913
11	61577	3065	48280	2688	25025	448
12	78641	2146	35723	1003	19683	2469
13	80688	1315	36866	2050	24096	2416
14	47840	3747	56492	2524	48807	5444

Counts on the OVPI jars after the removal of the uncrowded test Daphnia were 26,000 - 42,000 cells/ml, indicating that the Daphnia were not starved prior to the start of the incubation period. Size measurements on the test Daphnia at the end of the experiment are given in Table 4.

The heavy mortality of the algae in the CCW control was not anticipated and was quite startling. The algae were stable in the regular (RW) medium over the 12 hour period, with little or no mortality. The cells in the crowded conditioned CCW medium died off at a rapid rate, with a mortality of 59% in 12 hours. The counts are plotted in Figure 16 and listed in Table 5. A student's t test using pooled variance for the differences over 12 hours shows that the mortality is highly significant ($p < .001$) for algae in crowded Daphnia medium and no significant change in mortality in regular medium (RW) ($p < .5$).

The heavy mortality of the algae in the water conditioned by crowded Daphnia complicated the calculations of feeding rates for the uncrowded test Daphnia. Data for feeding rates in CCW had to be corrected for the mortality of the algae seen in CCW control jar C. Calculations of f and C_A were otherwise as previously described, and these values are given in Table 6.

Feeding rates were strongly repressed in Daphnia placed in CCW in the 6 - 12 hour interval. The feeding rates were

Table 6. Feeding rates for Daphnia at 30/liter in CCW and in RW media, corrected for mortality of algae in CCW medium.

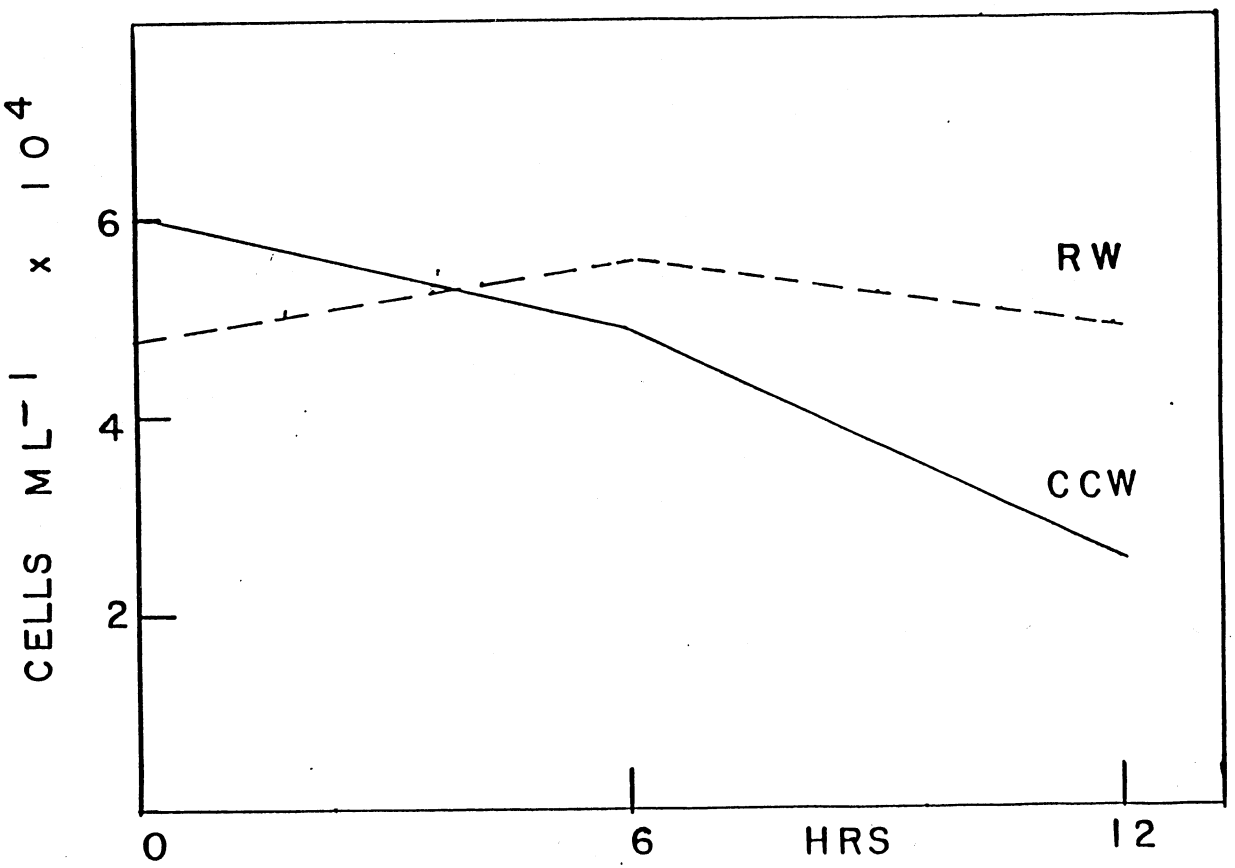
		0 - 6 hours		6 - 12 hours	
Jar	Medium	f	C_A	f	C_A
9	CCW	96950	35767	0	19357
10	CCW	72956	41569	0	24050
12	RW	238433	53003	89361	26486
13	RW	243456	58777	70944	29805

Table 7. Observed f rates compared with the rates expected as predicted by the Ivlev feeding function for 30/1.

		0 - 6 hours		6 - 12 hours	
		Obs	Exp	Obs	Exp
9	CCW	96950	64439	0	37740
10	CCW	72950	72869	0	45832
12	RW	238433	88104	89361	49884
13	RW	243456	95155	70944	55246

negative, perhaps there was not quite as much mortality in the Daphnia jars as in the control. In order to compare feeding rates made at different cell levels, one can calculate the rate expected for each concentration using the Ivlev function shown here. The function was derived from the

Figure 16. Mortality of C. reinhardi over time in medium conditioned by crowded Daphnia (CCW) and in regular medium (RW). All Daphnia removed before algae were added at time zero.


CELLS ML⁻¹ x 10⁴
6
4
2
RW
CCW
0
6
HRS
12

experiments on feeding rates for Daphnia at a density of 30/ liter:

$$f = 212639\ (1 - e^{-1.0094 \times 10^{-5} x})$$

Predictions of feeding rates using the Ivlev function are given in Table 7 as "expected" alongside the observed f rates. The uncrowded Daphnia feed close to the expected rate in the first six hour interval, and feeding ceases in the second six hour period. On the other hand, Daphnia in regular medium, RW, feed well above the expected rate. It is possible that feeding was already being inhibited in CCW in the first six hours but this possibility cannot be tested.

The source and nature of this potent inhibitor of feeding in crowded Daphnia water is unknown. The test Daphnia had been in CCW overnight before transfer to more CCW for the feeding incubation in the morning. All CCW had crowded Daphnia for at least 12 hours before use. One can speculate that the Daphnia somehow affect the medium and cause the algae to die, and that the dying algae in turn produce a substance that depresses the feeding rate of these Daphnia. This will be considered further in the concluding chapter.

Chapter 10
Summary and Conclusions

This discussion will cover first a summary and overview of the results, second, the density dependence in feeding rates in relation to food requirements for reproduction, third, three hypotheses concerning the observed depression of feeding rate in crowded Daphnia, and fourth, the advantages of low food for Daphnia and a model of the successful Daphnia population in lakes.

Summary of results

The cage experiments described in Chapter 3 provided the following results:

1. Reproduction in uncrowded test Daphnia was reduced in the presence of crowded Daphnia isolated behind a net barrier.

2. Growth was reduced in adult females and in young born in the presence of caged crowders and reared apart.

3. There was no stimulation of male production whether crowders were present or not.

4. The reduced reproduction was prevented by placing the crowders in dialysis tubing, indicating the effect could be caused by a large molecular weight compound or by particulate fecal matter or bacteria, but not by a low molecular weight compound such as NH_3.

5. The unexpectedly large decline in algal cells in the

medium with caged crowders was an effect of the crowders on algal mortality, as demonstrated later in Chapter 9 in the allelopath experiment, where medium preconditioned with crowded Daphnia caused greater mortality of C. reinhardi.

The reduction seen in reproduction in the presence of net-caged crowders could be the result of the observed reduced growth because of the strong dependence of egg production on body length. Since the experiments were designed to provide food in excess, the reduction in growth would either be the result of some factor depressing the feeding rate or the result of an effect on metabolism, e.g., by repression of oxidative metabolism. Depression of feeding rates in both crowded and uncrowded Daphnia by water conditioned with crowded Daphnia was demonstrated in Chapters 6 and 9, respectively. It is still possible that there are additional effects that could retard growth, e.g., from a molt inhibitor.

In the experiment on reproduction, with no change of medium, crowding stimulated a small percentage of male production compared with no male production in uncrowded Daphnia. Shifting the crowded Daphnia to fresh medium and low food caused a large increase in male production. In the future, a comparable shift should be made with low density Daphnia to see if a similar response in male production occurs. The high mortality in the low density cultures prevented such a shift in the present study.

To test the possibility that the high male production that resulted when the crowded cultures were shifted to low food was not actually caused by the food reduction, but rather by the shift to fresh medium that contained fresh vitamins and growth factors (D64), the following experiment should be run. Low and high density populations kept in the old medium would be shifted to low food levels. In addition, some of the jars of low and high density Daphnia kept in the old medium could be fed at the regular high level of food as before, but with and without a "spiking" of the medium with vitamins to see if males are produced and if there are density differences. In particular, α tocopherol should be added under these conditions. Birky and Gilbert (1971) found α tocopherol, normally present in green algae, to be essential for the production of mictic (sexually reproducing) female rotifers, and for male fertility.

Finally, the role of food levels in the shift to sexual reproduction should be clarified by very "fine tuned" measurements of food levels and male production, using a continuous flow system to maintain constant food levels. With this information, one could then determine whether the repressed feeding rates seen in the crowded Daphnia could reduce food intake enough to stimulate production of males in crowded populations. This might explain the effect of crowding on gamogenesis reported by earlier researchers (see Chapter 1).

Recently, D'Abramo (1980) reported that a rapid reduction of particle intake in Moina macrocopa stimulated male production, and that gamogenesis could be prevented by sufficient particle concentration in the medium, whether the particles were nutritive or non-nutritive. He felt the effect of crowding on sexual reproduction was the result of the rapid removal of particles by the crowded population and the ensuing low ingestion rate in reduced food.

In the reproduction experiment here (Chapter 4), mortality was high in the low density cultures, possibly because there was a daily carryover of roughly 50% of the algal food. Senescent algae can cause mortality in Daphnia (Ryther, 1954). The mortality in the crowded culture, on the other hand, was consistently low, even after the shift to low food. Low food has been observed by others to increase longevity in cladocerans because the metabolism is reduced. The spent Daphnia medium caused high mortality in fresh C. reinhardi over 24 hours. Young born into the old medium showed high mortality and aborted their eggs or produced no eggs. Reproduction was not depressed in these crowded Daphnia.

In studying feeding rates of D. pulex at three densities over a range of concentrations of C. reinhardi (Chapter 6), a strong density dependence of f was observed with a repression of the f rate of crowded Daphnia. Uncrowded Daphnia fed at very high rates. These data did not fit the Michaelis-

Menten function very well, but fit the Ivlev function extremely well (Chapter 8). A combined Ivlev function that includes the effect of Daphnia on feeding gave suitable back predictions to the original feeding rate curves for the three densities studied.

Finally, a complete repression of the feeding rate in uncrowded Daphnia was caused by medium that was preconditioned with crowded Daphnia (Chapter 9). Mortality of C. reinhardi was greater in the medium preconditioned with crowded Daphnia. One can speculate that the induced mortality in the algae is causing the reduction in the feeding rate in crowded Daphnia perhaps by the release of a toxic metabolite from the dying algal cells.

Density dependent feeding rates in relation to food requirements for reproduction in Daphnia

Without further study, it is difficult to state whether or not the degree of repression in the feeding rate of crowded D. pulex would depress growth and reproduction. Workers seeking the minimum food level required for egg production in cladocera suggest that food must be above 50 - 200 μ gC/liter (Duncan, pers. comm., Lampert and Schober, 1980), and further that maximum egg production in Daphnia will occur at food levels above 400 - 600 μ gC/liter. Translating these

values into equivalents of C. reinhardi cells, using the average value for C content of 0.02 μgC/cell (Porter, Gerritsen and Orcutt, in press), gives the prediction of maximum reproduction at 20,000 - 30,000 cells C. reinhardi cells/ml. The minimum for any reproduction would range from 2500-10000 cells/ml. On the other hand, Richman (1958) reported increasing reproduction in D. pulex fed C. reinhardi from 25,000 to 100,000 cells/ml. Hrbácková and Hrbácek (1978), rearing European D. pulex on "natural food" from ponds, achieved very low reproduction, 2.4 eggs/female, at their highest food level of 200 joules/liter, roughly equivalent to 38,000 cells C. reinhardi/ml. Clearly, this important area in cladoceran biology requires further investigation, if food level changes or changes in feeding rates are to be translated into effects on growth and reproduction.

Three hypotheses concerning the cause of the density dependence of f in Daphnia

1. Predation avoidance hypothesis

One can hypothesize that the reduction in feeding rate observed in crowded Daphnia is a side effect of behavior related to predator avoidance. The crowded Daphnia could sense their neighbors visually, by physical contact or by sensing the small currents generated by swimming and feeding

motions of the other Daphnia. The feeding current can be seen some distance (around .5 mm) from the anterior ventral edge of the carapace. They could also be sensing excretory compounds or other chemicals liberated from the other Daphnia. It is possibly a combination of all three stimuli.

The avoidance response could include the following, all of which are quantifiable:

1. reduced rate of swimming antennal movement
2. cessation or slowing of the swimming rate
3. a sinking response involving carapace closure, as has been observed in some chydorids, which can "clam up" and sink
4. reduction of all appendage movement to avoid detection

All of these actions, particularly 3 and 4, would result in reduced feeding activity. These rates could be compared in crowded and uncrowded conditions. (See work of Strickler, 1977, for measurement of swimming). The carapace gape and filter appendage rates could be measured using the method of Gliwicz (1980). One could add other species and test the rates in relation to density and interspecific interactions. Artificially generated small currents, e.g., with a vibrating fine needle or a "dummy" zooplankter, could be used to see if the Daphnia show any predation avoidance response stimulated by currents alone. To test for chemical signalling, one could measure various responses, carapace gape, appendage

and swimming rates, using water conditioned with crowded Daphnia. One could condition the water with other species of zooplankton and test for species interactions.

2. A hypothesis of population regulation

The crowded Daphnia sense each other either physically, by vision, by contact or by sensation of feeding and swimming currents, or chemically. The response would be a reduction in the feeding rate which in turn would reduce growth and reproduction. For this hypothesis to explain the density dependence in feeding rate observed here, the reduction in f would affect reproduction adversely. Contrary to prediction, the crowded Daphnia produced larger broods than the uncrowded Daphnia.

3. A hypothesis that the reduction in f is algal mediated

In this hypothesis, the algae somehow sense the crowded Daphnia and liberate a protective "anti-Daphnia" factor, or more likely, as the algae experience mortality in the crowded Daphnia medium they release a factor that depresses the feeding rate in Daphnia. The product released by the algae could cause an inhibition of nerve or muscle so gut peristalsis and gut passage times are reduced, thereby reducing feeding rate. It could also be a physical factor such as a mucilaginous

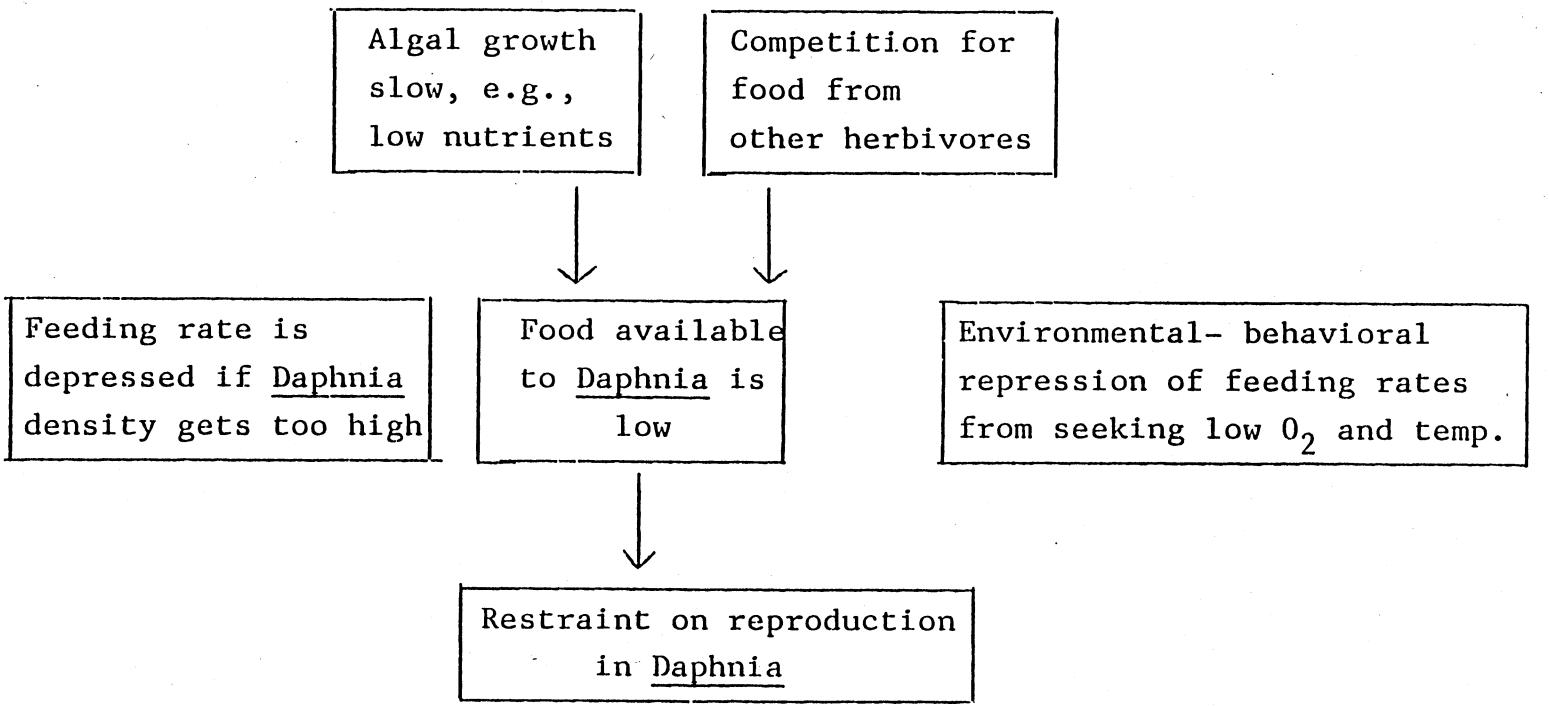
compound that would clog or adhere to the filtering apparatus, or a chemical compound that reduces muscular activity in the appendages. All these speculations are subject to experimental analysis.

The advantages of low food: the successful Daphnia population.

However it is mediated or caused, reduced feeding in Daphnia may have adaptive value in a natural environment. Daphnia are capable of feeding at very high rates and may achieve very high reproductive rates in the presence of ample food. Even though they can be found in nature occasionally at very high densities (over 700/liter, see Chapter 1) , and can be cultured at even higher densities (Slobodkin, to 14,000/liter!), such high densities are the exception. In the lakes, at least locally in Minnesota, where D. pulex dominates the zooplankton year around, they do not achieve densities much greater than 30 - 50/liter. The route to "success" for Daphnia pulex, then, may require some restraint on either its reproductive or its feeding capabilities, or both. One could postulage that anything that reduces the food intake is advantageous to D. pulex by preventing a temporary explosion and subsequent crash in the population. The model presented here outlines simply some of the factors that

could be acting in this fashion to limit the food intake and thereby create the success of this zooplankter in certain lakes.

It is important to note that Daphnia fed on a low food diet live longer than those on high food because of the reduction in metabolic rate as seen in the reduced heart rate in low food. The adaptive value of diel vertical migration, then, is not only the escape from predation resulting from remaining in the darker bottom water during the day, or the improved energetics from spending time in the colder bottom waters (McLaren, 1963), but also in the reduction in feeding rate brought about by the lower oxygen and temperatures in the hypolimnion of stratified lakes.


Algal growth slow, e.g., low nutrients
Competition for food from other herbivores
Feeding rate is depressed if Daphnia density gets too high
Food available to Daphnia is low
Environmental- behavioral repression of feeding rates from seeking low O_2 and temp.
Restraint on reproduction in Daphnia

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Appendix I Gorham's ASM-1 medium

	Final conc mM	Stock g|liter	Add ml/l
$NaNO_3$	2	17.002	10
$MgSO_4$	0.2	4.930	10
$MgCl_2$	0.2	4.065	10
$CaCl_2$	0.24	3.484	10
K_2HPO_4	0.10	1.742	10
Na_2HPO_4	0.10	2.681	10
$FeCl_3$	0.004	0.108	10
H_3BO_3	0.04	0.247	10
$MnCl_2$	0.007	0.138	10
$ZnCl_2$	0.0032	0.0436	1
$CoCl_2$	0.00008	0.0019mg	10
$CuSO_4$	8×10^{-7}	0.01516mg	10
Na_2EDTA	0.02	0.726	10

Appendix II. Effects on Daphnia fed C. reinhardi cultured with NO_3^- and high light.

In the earliest tests of the effects of food levels on reproduction and growth in D. pulex, the C. reinhardi cultured with NO_3^- as nitrogen source and under very high (3300 ft. ca.) light caused an inhibition of reproduction at the higher food levels. Usually 10 newborn Daphnia were placed individually in 20 ml D64 medium in vials at each food level studied. In one case, a complete inhibition of reproduction was seen at 400,000 cells/ml and above. In another run, reproduction was very poor at 100,000 cells/ml and above. In an experiment where food levels ranged from 500 cells/ml to 1.5 x 10^6/m;, mortality was high, 90%, in the two lowest levels (500 and 1000/ml) and reproduction occurred only between 10,000 - 100,000/ml, at 500,000 - 1.5 x 10^6/ml, no reproduction was seen, and the parent Daphnia at the higher food levels were pink, indicating the stimulation of hemoglobin syntheses. The color could not be removed by active aeration of the cultures. At the highest food levels, the pH ranged from 7.25 - 8.25.

Under the conditions of culture of C. reinhardi described above, the cultures tended to become somewhat yellow at high concentrations, and contained cells with vacuolations and shrunken chloroplasts. Clumping sometimes occurred under these conditions, whereas it did not occur in HSM-5 and low light. Changing media and lighting cleared up all these problems.

The severe inhibition of D. pulex reproduction (less on growth and survivorship) and the apparent stimulus of hemoglobin synthesis is unexplained. Proctor (1957) found that

Appendix II (con)

the fat-like extracellular metabolite produced by dying C. reinhardi and inhibiting to the alga Haematococcus was most strongly produced under conditions of high light, and NO_3^- medium. The factor was most toxic at pH 8.5, and not toxic below pH 7.5 (Proctor, 1957). The Daphnia in these tests sometimes had the filtering apparatus almost clogged with a mass of debris that contained bacteria and a net of fine non-motile filaments. It may be relevant that Proctor (1957) observed that dead or dying cells of C. reinhardi grown on NO_3^- contained a mucilaginous material that clogged ultra filters after only a few ml's passage. He also found that pH rose rapidly in cultures using NO_3^- whereas it did not with NH_4^+ as the N source, and that the cells grown in NO_3^- were yellowish. NO_3^- itself may be toxic to Daphnia (Taub and Dollar 1964). Ryther (1954) showed a depression of the feeding rate in D. magna fed senescent Chlorella vulgaris that ranged from a depression of 55% that of Daphnia fed growing cells at lowest food concentrations to a relative feeding rate of 11% at highest levels (Ryther, 1954).

These observations not only point to an interesting research project, but emphasize the need for further exploration of the dynamic interaction of the nutritional state of the algae with zooplankton reproduction, growth and mortality. The often hypothesized effects of "aging" algae in lakes in late summer on declining or crashing populations of zooplankton needs some real experimental work.

Appendix III. HSM (Sueoka, 1960) and HSM-5

	HSM	HSM-5		
	g/l	stock g/l	ml/l	final g/l
NH_4Cl	0.50	50	10	0.5
$MgSO_4 \cdot 7H_2O$	0.02	2	10	0.02
$CaCl_2 \cdot 2H_2O$	0.01	1	10	0.01
K_2HPO_4	1.44	-	-	-
KH_2PO_4	0.72	49.5	10	0.495
NaH_2PO_4	-	22.817	10	0.228
Na_2HPO_4	-	110.81	20	2.216
Huttner's	1 ml		1	
Vitamin mix			.5	

Vitamins

Primary stocks

1. Biotin 10 mg = 9.6 ml H_2O, 0.1 mg/ml final conc. Acidify slightly if autoclaving, freeze
2. B_{12} make 1 mg/ml, acidify slightly, autoclave, freeze.

Working solutions

1. Biotin 1 ml primary stock (.1mg/ml) plus B_{12} .1ml primary stock (1mg/ml), diluted to 100 ml, add 20 mg thiamine HCl. Autoclave in tubes and refrigerate. Add .5 ml mixture/liter medium before autoclaving.

Appendix IV. I/KI algae preservative per Sager

7.5 g I
7.5 g KI
Grind with 150 ml H_2O. Dilute to 250 ml and filter through Whatman #3 paper with suction. Can make in 95% EtOH. Keep cool.

Appendix V. D64 medium per Hosseinie (pers. comm.)

	mg/l final	g/l stock	add ml/l
Ca acetate·H_2O	22.0	2.200	10
$MgSO_4 \cdot 7H_2O$	4.095	0.4095	10
NaCl	20.0	2.0	10
KCl	1.0	1.0	1
$NaNO_3$	0.06	0.006	10
$Na_2HPO_4 \cdot 7H_2O$	0.03775	0.00377	10
$NaHCO_3$	8.0	0.800	10
$Na_2SiO_3 \cdot 9H_2O$	3.0	0.300	10
Ferric citrate	2.0	0.200	10
Ca pantothenate	2.0	0.200	10
B_{12}	0.0003	.1mg/l	3
thiamine	0.60	0.06	10
riboflavin	0.40	0.04	10
nicotinamide	1.3	0.130	10
folic acid	3.3	0.330	10
biotin	0.3	0.03	10
putrescine	0.3	0.100	3
choline chloride	5.0	0.500	10
inositol	11.0	1.100	10
Huttner's			1

Appendix VI. Embryonic stages, D. pulex

Stage*	Appearance
I	Eggs round, becoming oval
II	Eggs very oval, clear edges, first invagination
III	Egg membranes have been shed (not observed), embryo has bumpy surface, no cephalic development
IV	Cephalic crest formed, no eyes
V	Embryo with two red eyes
VI	Embryo with 2 darker eyes
VII	Embryo with single black eye
end	Embryo is mobile in brood pouch

*These stage numbers are those of Lei and Clifford (1974) used for D. schödleri. The longest stage in D. pulex is Stage I and II, around 24 hours at 20°. Hatching of the young followed by the molt takes at least one hour. Once the molt occurs, eggs are extruded into the brood pouch, undergoing considerable elongation as they make the sharp turn into the pouch.

Appendix VII. Conductivity measurements on various media

Specific conductance was measured using a Beckman Model RC 16B2 Conductivity Bridge with a Yellow Springs Instrument Cell 3402 (k = .1). Silver nitrate standards of known normalities were measured and used to calculate C, the cell constant, by multiplying the resistance R in ohms times the specific conductance in reciprocal ohms/cm for each concentration of standard. Specific conductance of samples is then obtained in mho/cm by dividing $\frac{C}{R}$, or cell constant by measured resistance in ohms, the result is converted to micromho/cm. The cell constant of the standard closest to the measured sample resistance was used, but all three constants were close to 1. Results are given below:

Specific conductance of old Daphnia medium and culture water. Temp. 18°.

	micromho/cm		micromho/cm
D64, aver	1136	glass dist H_2O	26.3
10-1	1325	" " "	11.6
10-2	1380	", fresh poured	8.9
10-3	1325	fresh tap H_2O	1438
10-4	1329	aged tap H_2O	2096
10-5	1343	stock tank	3999
10-6	1338	HSM, sterile	24383
Aver. 10's	1340	Algae culture, ca..5 x 10^6/ml	25271
270-1	1746	Algae culture,	
270-2	1740	ca 2 x 10^6/ml	25658
Aver. 270's	1743		

The aged tap water had Na thiosulfate added. Most U.S. waters fall in the range of 50-500 micromhos/cm up to 1000 in highly mineralized water (Standard Methods, 1971). The average for Swedish lakes is about 406 (Rodhe, 1949).

Appendix VIII MM medium used in early feeding rate study.

	g/liter
NH_4Cl	0.05
$MgSO_4 \cdot 7H_2O$	0.02
$CaCl_2 \cdot 2H_2O$	0.01
KH_2PO_4	0.2475
NaH_2PO_4	0.114
Na_2HPO_4	1.108
Huttner's trace metals	1.0 ml

Appendix IX. In vivo fluorescence measurements.

In vivo fluorescence of algae was related to cell counts using the Turner Model 111 Fluorimeter with a photomultiplier sensitive to 300 - 700 micrometers, but falling off rapidly at 600 micrometers and above. This means the fluorimeter can only respond to the lower wavelength part of the chlorophyll fluorescence emission spectrum. The red sensitive photomultiplier that is necessary to detect the entire spectrum was not available. High correlations were obtained with cell counts and F readings on living C. reinhardi cells, r = .979, as well as from F readings on living cells treated with DCMU (r = .959) to allow maximum fluorescence by blocking transport of electrons from chlorophyll a. The in vivo regression of F readings converted to a 1x scale as y against cells/ml as x is:

$$y = (2.517 \times 10^{-5})\, x + 2.790$$

The use of the herbicide DCMU or 3-(3,4-dichlorophenyl) -1, 1-dimethylurea, at 10^{-5} M to cause maximum fluorescence of chlorphyll a doubled or tripled the F readings. The extra step is recommended because of daily variation in the photosynthetic activity of the chlorophyll molecule (see Samuelsson, Oquist and Halldal, 1978). The fluorimeter can detect very low cell levels, around 1000 cells/ml if the 30x scale is used, but there was no improvement in error range.

Appendix X. Translation of Richard's (1896) descriptions of the Daphnia pulex forms, pp. 232-5, 237-41.

Daphnia pulex Leydig hatata

Body large, heavy. Head short, low, comprising around 4,5 times the body length, without the caudal spine which is relatively long. Ovigerous females attain or surpass 3mm in length. The anterior edge of the head is a little convex. The ventral edge has a distinct sinuosity that is not very deep. The rostrum is well developed, acute or pointed, and bent back. The forehead is not very prominant. The fornix is high, beyond the eye. The head is separated from the body by a weak dorsal impression hardly perceptible.

The eye is large, with crystalline lenses that are not very numerous and poorly disengaged with pigments. The eye-spot is small, nearly as removed from the eye as from the posterior edge of the head. The anterior antennae are similar to those described.

Daphnia pulex Leydig

Body large in the middle, heavy enough in appearance. Head short, a little low, comprising around 4,5 times the total body length (without the caudal spine which is short). Ovigerous females may have from 1.8 - 2.5 mm length. Anterior edge of the head a little convex. Ventral edge has a well marked and often deep concavity. Forehead is prominent. Rostrum well developed, pointed, directed to the rear, often strongly recurved. Fornix high, going just beyond the eye. Head separated from the body by a dorsal impression that is very weak, often hardly perceptible.

Eye is large, with few crystalline lenses, medium

disengaged with pigment. Medium eyespot, nearer the posterior edge of the head than the eye.

Anterior antennae very small; inside of them and on a median line, the posterior surface of the head forms a prominance or bulge . The extremity of the short sensory bristles almost reaches the extremity of the rostrum.

The posterior antennae attain or pass a little the middle of the valve length. The small groups of cuticular thickenings simulating spinules and arranged in annular series on the joints of the antennas are as well marked as the teeth of the distal extremity of the joints. The last two joints of the ventral branch carry long hairs on their dorsal face. The bristles are long and strongly ciliated.

The valves of the carapace are around 1.2 times longer than wide. The dorsal edge, convex enough, is spiney in its last half, as well as the ventral edge which is more convex. The inner edge of the ventral edge has in its posterior part short slender hairs, difficult to see. The two edges are reunited in a short caudal spine whose origin, situated always above the longitudinal axis, can nevertheless be brought nearer. This caudal spine hardly surpasses a tenth of the body length of ovigerous females, it can be reduced to a simple prominence: it is often longer in ephippial females and can then attain twice the previously indicated length. Reticulation of the valves is well marked with large enough meshes, square or rectangular.

Gastric caecum short and recurved at the free end.

Of the four abdominal extensions, the first is pointed, twice as long as the next one, supplied solely with some scattered hairs, delicate, the others are very distinctly haired. The fourth is reduced to a slight prominence. The postabdominal bristles, ciliated, do not pass much more than half the distance which separates their source from the origin of the terminal claws.

The postabdomen has 12-15 slender teeth, lightly incurved, pointed, gradually diminishing along their length. One observes a very distinct sinuosity on the dorsal edge following the teeth. The sides of the postabdomen are supplied, in their proximal part, with small spines arranged in groups. One observes, in addition, a series of very fine spinules along the dorsal edge, near the origin of the teeth. The terminal claws carry two small notches in their ventral edgs and two combs of which the proximal has 4 to 8 teeth smaller than those of the distal comb which has 6 - 9.

The male attains 1.4 mm length, without the caudal spine which can measure .34 mm. The head is rounded, without a rostrum, its ventral edge is straight, often lightly convex; very rarely it has a very slight sinuosity. Its anterior edge is a little convex. without an impression. The anterior antennae do not attain a third of the length of the head (taken from the breadth of the origin of the antennas). Flagellum is not ciliated, attains around 2/3 of the length of the antennae. Sensory bristles almost as long as the basilary joint of the flagellum. A small bristle originates on the anterior edge of the antenna in its last quarter, at a distance from the extremity of this equal to the width of the antenna at this point. The abdomen has one distinct extension, very long, pointed, attaining almost 1½ times the length of the anterior antennae. (It measures .32mm in a male of 1.3mm). This extension is supplied with small short fairly distinct spiniform hairs, disposed in an annular series. The dorsal edge of the postabdomen has a strong sinuosity following the teeth which number around 11 and which diminish gradually along their length. The proximal comb of the terminal claws has around 7 fine and small teeth, and the distal claw has 6 stronger teeth. Reticulation is well marked with large square meshes.

Daphnia pulex Leydig, var. pulicaria, Forbes

Body is small in the middle, measuring around 1.9 mm without the caudal spine. Head short, low, anterior edge of the head is a little convex. Ventral edge is widely concave. Rostrum is long, pointed, directed backwards against the anterior edge of the valves. Fornix goes as far as the eye. Dorsal impression generally absent, but sometimes well marked in ephippial females.

Eye large, its vertical diameter is scarcely contained two times in the distance which separates the extremity of the rostrum. It is situated near the frontal edge, rounded and provided with large and numerous crystalline lenses. A medium eye spot is situated at equal distance from the eye and the posterior edge of the head.

The anterior antennae with sensory bristles does not attain the extremity of the rostrum. Joints of the posterior antennae are supplied with fine hairs arranged transversally and are not very visible.

Natatory bristles are medium, ciliated enough, with 3 joints of which the third is very short but distinct.

Valves are oval. Dorsal and ventral edges furnished with spines in their posterior half, smooth in the rest of their length. Caudal spine is of variable length, situated on the dorsal edge (side) of the longitudinal axis and attaining around one fourth the body length. Reticulation is well marked, with quadrangular mesh.

Gastric caecum recurves strongly towards the extremity.

Of the four abdominal projections, the first two are contiguous at their base; the first and the longest ones are smooth, the others are ciliated. The last two are less remarkable. The postabdomen is large enough and carries at its dorsal edge 13-17 teeth diminishing gradually along the length. The terminal claws have two indentations at their

ventral edge and two combs formed of 6 teeth each, the proximal ones being smaller. The length of the adult female is 1.9 mm, width 1.0 mm, caudal spine .5 mm.

The male reaches 1.4 mm without the caudal spine which is .33 mm long. The head is straight, its ventral edge lightly concave forming an obtuse rostrum just underneath the insertion of the anterior antennae. These are short, not attaining a third of the width of the head, lightly swollen towards the middle of their length. Flagellum curved in, pointed, as long as the antenna itself. Sensory bristles notably shorter than the basal joint of the flagellum. Accessory sensory bristle situated on the anterior edge of the antenna, a little near the last third. The abdomen has one distinct projection, ciliated, a little pointed, almost as long as the anterior antennae. The dorsal edge of the postabdomen has a strong sinuosity following the teeth which number 12-14 and are of decreasing length.

Observations. The preceding description has been made after the text and drawings of Forbes. Birge has given several figures of this variety which agree well in general with the original description. As remarked by Forbes himself, the var. pulicaria resembles a lot the D. pulex type, especially in the females. The males present more marked differences, although in sum weak enough. Birge compares pulicaria with schödleri; there are in effect a number of common points in the two forms, but the original difference of the caudal spine permits separating them clearly.

Geographic distribution: U.S. Yellowstone Lake and nearby waters (Wyoming), Wisconsin. This form appears to be a variety of D. pulex adapted to pelagic areas of lakes. Birge has found near Madison specimens which compare apparently to this variety and which live in temporary ponds where they lost part of their transparency. In L. Mendota (Ill.) this variety is as hyalin as D. hyalina.

Daphnia pulex Leydig var. Minnehaha, Herrick

The adult female of this variety is not distinguished by any character well marked and constant of the typical form, and there is no space to give a special description of it; the general form of the body, the structure of the anterior antennae, the mesh relatively large and well marked on the carapace, the long abdominal projection of the male are similar in the two forms. But in the young individuals of the two sexes one observes a prominence in the crest of the dorsal edge of the head, a prominance terminated by denticulations which number from 1-5. This denticulated projection and the number of denticulations diminishes at each new molting, such that there remains no trace in completely developed individuals.

Fischer first mentionned and drew this feature. Typical adults do not pass 2.5 mm in length, the dorsal prominance having 1-5 teeth in the two sexes and disappearing completely in the adult.

In 1884 Herrick observed the same variety and named it Daphnia minnehaha. The number of teeth on the dorsal projection is 1-4. After Herrick, the adult male such as it is figured, has 4 teeth and the abdominal projection is shorter than that of D. pulex and deprived of the hairs that one observes in this last form....Let us note finally that after the drawings of Herrick the dorsal projection is situated more to the rear than in European specimens, the head of the adult female appears also longer and the ventral edge of the head is less excavated..... Geography: France, Russia, Minnehaha Creek, Minnesota, Madison, Wisconsin.

Daphnia pulex Leydig Schödleri

The body is slim in appearance. Length 1.15 - 1.6mm, (spine .48 - .56 mm). Head long enough, low, having scarcely

4.1 times the body length. The ventral edge of the head is almost straight or presenting a very light sinuosity. The anterior edge is convex. The rostrum is well developed, directed to the rear. Fornix high, going as far as the eye. The front is a little prominent. Viewed from the side the head is enlarged by the crossing of the rostrum. The dorsal impression, hardly noticeable, is moved back towards the middle of the body length.

The eye is large, approaching the frontal edge, with crystalline lenses numerous enough and well disengaged with pigment. The eyespot is small, more near the posterior edge of the head than the eye.

The anterior antennae as in D. pulex. Posterior antennae pass the middle of the length of the valves. The linear cuticular projections, grouped in annular series, and which simulate spinules at the surface of the joints, are extremely delicate and so fine that they easily escape the eye. The denticles of the distal extremity of the joints are not very numerous and small. Natatory bristles weakly haired.

Valves of the carapace 1.4 times longer than wide, oval. Dorsal edge a little convex, it is often less than the posterior edge of the head, which gives the animal an aspect very particular. The dorsal edge is supplied with strong spines until the beginning of the dorsal impression, that is to say, until the traverse of the middle of the body length. The ventral edge is scarcely more convex than the dorsal edge; it is spiney in its posterior half. The hairs of the inner rim are very short and very thin. The two borders of the valves are united under the median line in a long caudal spine, attaining around half the length of the valves. It is thin, supplied with strong spines and lightly incurved at the dorsal side. Reticulation of the valves is very distinct with mesh relatively large more or less regularly square.

Caecum medium, at end strongly recurved.

Of the four abdominal projections the first, which would be bare is not much longer than the next one which is haired strongly enough as much as the other two. The third is a projection distinctly rounded, the fourth is very small and very little projecting. The postabdominal bristles are like those in D. pulex. The postabdomen has ten thin teeth, pointed, lightly incurved, with length gradually decreasing. The postabdomen is conical, of medium size and carries on its sides only rare or weak spinules. The terminal claws have two small indentations at their ventral edge; the stronger proximal comb has 6 teeth and the distal 4-5 stronger teeth. One can observe along the dorsal border groups of extremely fine spinules towards the origin of the postabdominal teeth.