INGESTION, ASSIMILATION, SURVIVAL, AND REPRODUCTION BY *DAPHNIA PULEX* FED SEVEN SPECIES OF BLUE-GREEN ALGAE[1, 2]

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ABSTRACT

Daphnia pulex (Crustacea, Cladocera) was fed the blue-green algae (Cyanophyceae) *Anacystis nidulans*, *Synechococcus elongata*, *S. cedrorum*, *Merismopedia* sp., *Anabaena flos-aquae*, *Synechocystis* sp., and *Gloeocapsa alpicola*. The green algae (Chlorophyceae) *Ankistrodesmus falcatus* and *Chlorella vulgaris* were used for comparison. Direct observations were made of *D. pulex* feeding in depression slides filled with the test food. Food labeled with ^{14}C was used to determine ingestion and assimilation. Life tables were constructed for cohorts fed blue-greens, greens, and no food, and survivorship (l_x), net reproductive rate (R_0), median age of death, and intrinsic rate of natural increase (r) were calculated.

In all cases, ingestion, assimilation, survivorship, and reproduction of *D. pulex* fed blue-green algae were lower than of those fed green algae, although there were differences among the blue-greens in their effects on these parameters. *Anacystis nidulans*, *Merismopedia* sp., and *Synechocystis* sp. showed some toxicity or inhibition towards *D. pulex*. Although some blue-green algae can be ingested and assimilated by *D. pulex*, few if any of those tested provide sufficient nutrition to support a population that does not have other food available.

INTRODUCTION

Eutrophication often results in a proliferation of blue-green algae, many of which can displace other species by forming heavy blooms. This study investigated the effects of such situations on zooplankton which normally feed on planktonic algae. The toxicity of several blue-green genera to higher animals is well established (e.g., *see* Gorham 1965; Shilo 1967), but only a few tentative reports of toxic effects of blue-greens on aquatic invertebrates exist (Braginskii 1955; Dillenburg and Dehnel 1960; Smirnov and Fooklistova 1963; Vance 1965; Stangenberg 1968; Gentile and Maloney 1969). In cases where zooplankton are unable to feed on the predominant phytoplankton, or where their populations have been depressed by previous unsuitable food conditions, grazing could cease to be a factor in the control of phytoplankton populations (as it usually is: *see* Edmondson 1957). This could contribute to the development of nuisance conditions.

It is commonly believed that Cladocera feed on phytoplankton, bacteria, and organic detritus, but many reports of exceptions exist. Edmondson (1957) has reviewed the subject in detail. The Cladocera are obligate filter feeders, having appendages specialized for respiration and food gathering, as well as for some degree of food selection and rejection. Although some forage on submerged surfaces, most genera, including *Daphnia*, filter the open water. Food is rejected when the amount collected is greater than can be ingested, when it is physically unacceptable (e.g., colonies or filaments too large), or perhaps if it is chemically unacceptable (Burns 1968). Undesirable food may also be passed through the gut without digestion, but little evidence for or against this has been published (Edmondson 1957; Fitzgerald 1964).

Lefèvre (1942) studied the food value of various species of phytoplankton to Cladocera, testing each species by determining

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its presence in the digestive tract and by observing the growth of populations of Cladocera fed pure cultures of the alga; I have used modifications of the same techniques. Although his early study did not include blue-greens or diatoms, he later (Lefèvre 1950) reported that *Aphanizomenon* was unsuitable as a cladoceran food. Sorokin et al. (1965; Sorokin 1968) concluded that *Aphanizomenon flos-aquae* and *Coelosphaerium dubium* are adequate foods, but that some blue-greens such as *Microcystis aeruginosa* and some species of *Anabaena* are not utilized to any extent. Schindler (1968) found that *Anabaena* was assimilated by *Daphnia magna* at a much lower rate than were *Chlamydomonas* and *Chlorella*. Others (Birge 1898; Monakov and Sorokin 1961; Hall 1964; Edmondson 1965; Burns 1968) have questioned the suitability of blue-greens as food.

The ability of the Cladocera to use a variety of foods makes interpretation of the effects of one particular food in natural populations difficult (Brooks and Dodson 1965). Death due to toxicity of food was suggested by Gentile and Maloney (1969) but is relatively unlikely in most field situations. Death from starvation is equally unlikely, as Cladocera can live for extended periods on little food. Cladoceran populations can thus be expected to respond to food conditions principally by changes in reproduction.

Reproduction and growth in Cladocera are related since release of young and increase in size both take place at molting. Normally, a new brood is released at every molt from the fifth instar (Anderson et al. 1937) until the last, which is usually sterile (Banta 1939). The food level affects the number of young per brood and the amount of growth per instar, which are significantly correlated (Anderson et al. 1937). Thus we would expect poorly nourished cladocerans to have slow growth and low reproduction as compared with well-nourished ones kept under otherwise identical environmental conditions (Beerstecher 1952). This assumption is basic to the life-table evaluations below.

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MATERIALS AND METHODS

Blue-green algae

The following cultures of blue-green algae were obtained on soil-water medium from the Cornell University Culture Collection. (Taxonomy according to the lists of the Cornell and Indiana University Culture Collections; *see* Starr 1964.)

1) *Anacystis nidulans* (I. U. 626), very small cylindrical cells with rounded poles, length about twice the diameter (3–3.5 × 1–1.5 μ), cells occur separately in turbulent culture, with a sheath.

2) *Synechococcus elongata* (I. U. 563), small ellipsoidal to cylindrical cells with little sheath, often in pairs end to end. Length 15–20 μ, diameter 8–12 μ.

3) *Merismopedia* sp. (C. U., isolated from nature by H. Wiltberger), small ellipsoidal to spherical cells, 3–4 μ in diameter, singly or in pairs in turbulent culture, with a sheath.

4) *Synechococcus cedrorum* (I. U. 1191), very similar to *S. elongata*, tending toward spherical cells, also a pigment difference (*see* Gassner 1962).

5) *Anabaena flos-aquae* (I. U. 1444), cells cylindrical to barrel-shape (4–6 × 5–6 μ), in turbulent culture occurring singly or as short chains of three or four cells.

6) *Synechocystis* sp. (C. U., isolated from nature by F. J. DeNoyelles, Jr.), small spherical cells, 5–6 μ in diameter, tending to small clumps even in turbulent culture, no sheath. Probably is *Synechocystis aquatilis*.

7) *Gloeocapsa alpicola* (I. U. 589), colonies of a few spherical cells in a common sheath. Cells 1.5–3 μ in diameter.

The algae were maintained on soil-water culture in sterile 250-ml flasks until needed. Transfers were then made in a sterile hood to ASM-1 medium (Gorham et al. 1964) in

sterile 250-ml erlenmeyer flasks. Soil-water cultures were kept in dim light (409 lux), and liquid cultures were kept on a shaker table in bright light (4,482 lux) from "cool-white" fluorescent tubes. All cultures were kept on a cycle of 16-hr light and 8-hr darkness, at a temperature near 20C. Shaking was continuous at 185 cpm, and a few glass beads were added to each flask to disrupt any algal clumps that might adhere to the flask despite shaking (Taub and Dollar 1965).

Comparison foods

The following foods were tried at various times:

1) *Ankistrodesmus falcatus v. acicularis* (I. U. 101), crescent-shaped cells occurring singly in turbulent culture, fairly large (2.5 × 40–50 μ), no sheath.
2) *Chlorella vulgaris* (I. U. 30), small spherical cells, 5–9 μ in diameter, occurring singly, no sheath.
3) *Chlorella pyrenoidosa* (I. U. 26), same as above.
4) *Chlamydomonas* sp. (I. U. 206), spherical small (5–10 μ in diameter) free-swimming cells, no sheath, phototactic.
5) Yeast, Fleischmann's baking, suspended in distilled water, species unknown. Cells spherical, 10–15 μ in diameter.

The algae were cultured in the same way as the blue-greens. *Ankistrodesmus* proved simplest to culture and was used as the reference food. It was also grown in Bristol's solution for feeding stock cultures of *Daphnia pulex* before they were used in experiments.

Preparation of food suspensions

Suspensions of food at the desired concentration levels were prepared by selecting a culture which was near, but not at or beyond, the stationary phase of growth. This could be determined readily as the onset of the stationary phase coincided with a rapid color change from blue green to dark grass green. The optical density of the culture was then measured in a Klett-Summerson photoelectric colorimeter with a No. 42 blue filter. A limited number of cell counts of diluted suspensions of the algae were made and no great deviation from linearity between cell count and Klett reading was found; 16 Klett units equal about 1×10^6 algal cells/ml (Hall 1964). Optical density was the most convenient way of equating different foods for this study.

The Klett reading was then substituted into the formula:

$$K_d\ V_d/K_c = V_c,$$

where K_d = the Klett reading desired in the final food suspension; V_d = the volume of final food suspension required; K_c = the Klett reading of the stock culture; V_c = the volume of culture which, when made up to V_d, will give K_d.

Two food concentrations were chosen for use in the experiments: 25 Klett units to correspond roughly to a heavy bloom in nature, and 1 Klett unit as a compromise between more usual concentrations in nature and a level that would give detectable results in a reasonable time. Only the K = 25 level was used in the direct observation and radiotracer experiments so that an excess of food was available and counts were sufficiently greater than background. The centrifugation step used by Hall (1964) and others was omitted to prevent cell damage and the possible release of toxins from the blue-greens. This omission was probably not harmful; since the cultures were used near the end of the log phase of growth most of the nutrient salts in the medium that might affect the *Daphnia* were bound up in algal cells and the dilution necessary to get the desired final concentration was relatively great (from 5 to more than 20×). Also, ASM-1 medium was checked for toxicity to *Daphnia* by comparing survival of groups of about 10 individuals in serial dilutions of the medium with that of similar groups in sterile water over a 5-day period. No mortality occurred in either group.

Dilution water

Water for diluting the algal cultures to make food suspensions was obtained by

passing chlorinated tapwater (originating in Cayuga Lake) through an activated-charcoal filter to remove chlorine and some organic material and storing it in a stainless steel tank, from which it came through PVC plastic pipe to the laboratory. The water was quite hard, so it was diluted with one part distilled water to four parts treated water to reduce the formation of salt deposits on culture vessels upon evaporation. The mixed water was autoclaved in 4-liter flasks at 1.2 atm, 121C for 30 min, allowed to cool, and passed through an IIA (0.45 μ) Millipore filter into a sterile storage flask equipped with a delivery tube. The filters were washed with warm distilled water to remove detergents (Cahn 1967) before use.

Daphnia pulex *Leydig*

This species is widely distributed in the open-water plankton and occurs in both ponds and lakes. Samples were collected from Oneida Lake, New York, during summer 1968 and established in large aquaria in the above water mixture. I ran preliminary survivorship experiments in the prepared water, with and without autoclaving; survival was considerably better in the autoclaved water (Taub and Dollar 1968).

Several clones were started from individual adult females in battery jar aquaria (ca. 4 liter) with constant aeration and the same light-dark cycle used for the algae cultures. All animals for the life-table and tracer experiments were taken from two clones that originated with females from a single brood and were assumed to be genetically identical (Slobodkin 1954). All cultures were kept at 20C. The stock cultures of *Daphnia* were fed every other day on *A. falcatus*, but bacteria and detritus also developed in the tanks and undoubtedly contributed to the food supply. Experimental animals were allowed to empty their guts in sterile filtered water (with two intermediate rinsings) for at least 1 hr before use. All transfers were made with a large-bore medicine dropper to avoid injury to the animals. Droppers were flame sterilized to avoid unnecessary contamination of media.

Direct observations

Observations of *D. pulex* feeding on unialgal food suspensions (concn K = 25) were first made by a modification of the technique of McMahon and Rigler (1963). The bottom of the depression in a 1-ml clear glass spot plate was smeared with petroleum jelly, and individual adult female *Daphnia* were mounted for observation by placing them in the depression in a drop of water, then gently pressing their dorsal surfaces into the jelly with a probe. The water was then replaced with food suspension and the plate viewed by transmitted light under a dissecting microscope. Although the animals seemed to feed with no difficulty in this position, some variability in response may have been partly due to the unnatural orientation. A simpler, less variable method was therefore added. The animals were allowed to swim freely in the full depression, and when observations were to be made, the food suspension was removed with a micropipette to immobilize the animal. The liquid could be drawn down to a thin film covering the animal for 1 min or more without affecting its subsequent behavior; all animals immediately resumed swimming when the liquid was replaced with fresh food suspension.

Observations were made at 10-min intervals at magnifications of 25–50×. Between observations, plates were moved off the microscope stage into subdued lighting to minimize photic effects. Filtering activity, ingestion, rejection, and filling of the gut with food could be observed and timed easily using these techniques. Percent of the gut filled in 1 hr was estimated by observation and averaged for six individuals. No correction was made for possible variations in compaction of food in the gut.

Radioactive tracer experiments

Phytoplankton labeled with ^{14}C-HCO_3 has been used frequently as food in studies of zooplankton feeding (e.g., *see* Rigler 1971). I used the following version of the general method: 10 μCi of ^{14}C-HCO_3 were added to 1,500 ml of algal suspension at the K = 25 concentration and the suspen-

sion allowed to stand under room light with frequent stirring for 1 hr, during which ^{14}C was taken up by the algae. Meanwhile, two groups of 25 adult female *D. pulex* were isolated in sterile water and allowed to stand for 1 hr to ensure that their upper guts and food-gathering apparatus were free of food. At the end of the hour, 100-ml portions of the food suspension were measured into each of 10 old-fashioned glasses (200-ml capacity), which make ideal experimental vessels because they have no interior corners where food can accumulate. A 50-ml portion was filtered onto a Millipore HA filter (0.45-μ pore size) for determination of the prefeeding radioactivity of the algae and another 50 ml at the end of the feeding period to determine the average radioactivity in the algae during feeding. Samples of radioactive culture medium without algae and algal suspension without added radioactivity were also filtered. Five *D. pulex* were then added to each of the 10 glasses and allowed to feed on the radioactive algae for 1 hr.

At the end of the hour, *D. pulex* from 5 of the glasses were rinsed twice with sterile water, transferred to a suspension of *A. falcatus* with no label at a concentration of K = 25, and allowed to feed in it for 2 hr. This removed all radioactive food from the gut (except for a negligible amount that may have been refiltered from fecal material) and ensured that any remaining radioactivity in the animal was due to assimilated ^{14}C. The *D. pulex* from the other 5 glasses were given an anaesthetic rinse of club soda (a supersaturated solution of carbon dioxide in water) to prevent defecation and other removal of food from the gut (Rigler 1971), then a rinse of 1% hydrochloric acid to remove any radioactive food adhering to the exterior, then two distilled water rinses, and finally placed in a liquid scintillation counting vial. A few drops of Formalin-aceto-alcohol preservative (Pennak 1953) were added to the 50 ml of labeled algae filtered at the end of the feeding period to avoid additional uptake of radiocarbon during filtration. All filters were transferred to liquid scintillation vials after filtering.

The *Daphnia* in the unlabeled *Ankistrodesmus* suspension were removed at the end of 2 hr and treated as described above. Samples (50 ml) of the unlabeled food, taken before and after feeding, were treated like those from the labeled samples. Surplus water was then removed from all vials with a micropipette, and the vials containing algae were placed in a desiccator at 3.6-kg vacuum for 24 hr at room temperature. The vials containing *Daphnia* received 2 ml each of NCS reagent (a quaternary amine hydroxide which dissolves all the soft tissues and renders the hard carapace transparent), after which they were capped and held for 60C for 24 hr. A solution containing 4 g PPO + 0.5 g POPOP per liter of toluene was then added to fill each vial. Vials were stored in darkness until they were counted in a liquid scintillation counter at room temperature, with quench correction by the channels-ratio method (Wang and Willis 1965). Counting efficiency averaged 65%. The tabulated figures are means of three 10-min recounts, separated by at least 1 hr to allow for any self-excitation or other effects that might change with time.

Difference between means was tested by the "Student's" *t* test (Steel and Torrie 1960). Corrected counts of the *D. pulex* that had been given only labeled food were used as a measure of ingestion, while counts for those that had been flushed in unlabeled food were used as a measure of assimilation. There was a variation of from 0.5–62% in the amount of radioactivity taken up by the different algal species during the hour before feeding began. To correct for this, *A. falcatus* was taken as the standard, and the counts for the other experiments were adjusted up or down by whatever percent deviation existed between the uptake of the standard and experimental food suspensions. The uptake by *A. falcatus* was equivalent to 5,116 dpm/50 ml; that by *A. nidulans*, for example, was equivalent to 3,584 dpm/50 ml, and thus all counts involving *A. nidulans* were multiplied by 1.427. Variation in the counts of the algae at the end of the feeding period was considerably less than when uncorrected.

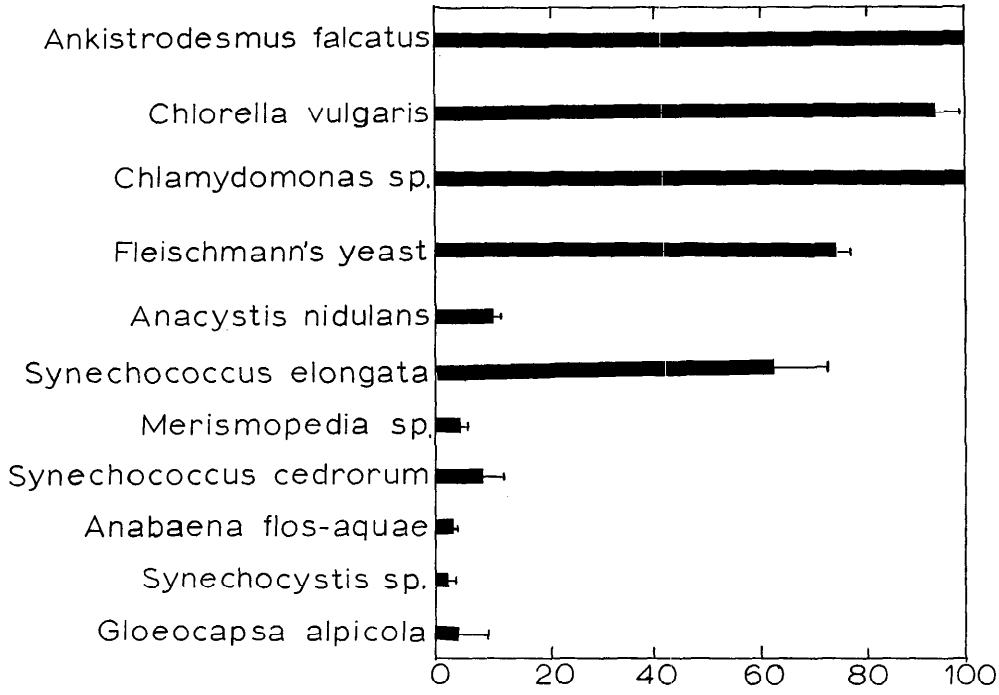


FIG. 1. Estimated percent of gut filled in 1 hr by *Daphnia pulex* fed unispecific food suspensions (concn K = 25). Average of 3 free and 3 mounted individuals. Standard deviation indicated by thin extension of bar.

The radioactivity of samples of unlabeled algae and media without algae was either zero or insignificant in every case.

Life-table experiments

Beginning with a newborn cohort, a life table states the number of deaths, the survivors remaining, the rate of mortality, and the expectation of further life for each age interval, symbolized by d_x, l_x, q_x, and e_x, respectively, where x represents age (Deevey 1947). The life tables constructed in this study include survivorship (l_x), age-specific reproduction (m_x), net reproduction rate or rate of population increase per generation ($R_0 = \Sigma\, l_x m_x$), intrinsic rate of natural increase (r), and median age of death. These parameters are often used as indices of zooplankton population conditions (Anderson et al. 1937; Edmondson 1957; Hall 1964).

Data were obtained from cohorts of 15 newborn *Daphnia* (<24-hr-old), each individual raised in a separate glass as described above. Glasses containing one cohort were kept together on a tray in a constant-temperature chamber at 20C, again under 18-hr light and 6-hr dark. To keep young individuals from becoming caught in the surface film, the tray bottoms were lined with black matte paper, and the trays were illuminated by dim overhead light; the *Daphnia* avoided the surface most of the time, without collecting on the bottom of the glass. Every second day the animals were transferred to fresh medium in a clean glass. Any newborn were removed after being counted with a hand lens with the glass immersed in a dark-bottomed water bath to minimize reflections; this method proved much faster than counts using a microscope or a pipette, and as accurate. There was no evidence that more than one brood was produced between changes, confirming the findings of Frank et al. (1957) that the gestation time of *D. pulex* in this temperature range was not less than 48 hr.

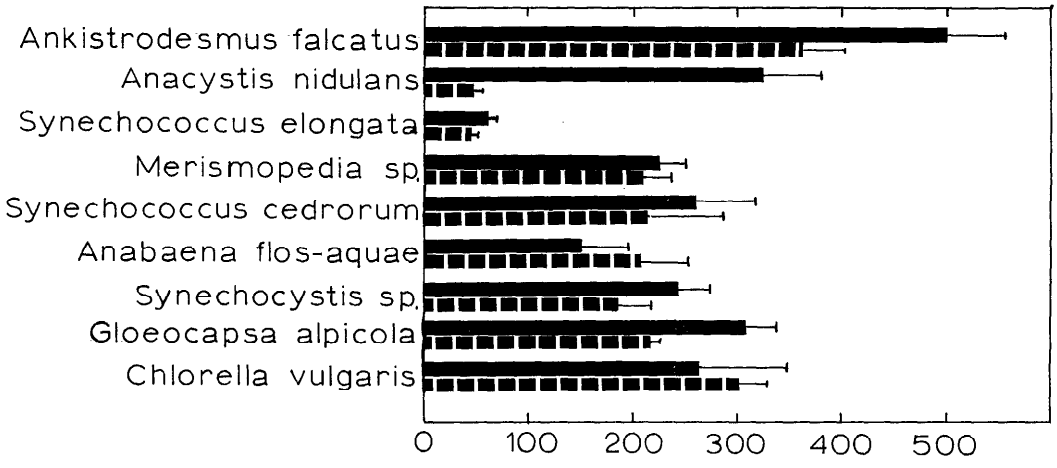


FIG. 2. Ingestion (solid bar) and assimilation (broken bar) of ^{14}C-labeled blue-green and green algae at concn K = 25 by *Daphnia pulex* fed for 1 hr, expressed as disintegrations per minute. Each bar represents the mean of 5 groups of 5 individuals each. Standard deviation indicated by thin extension of bar.

The parameters listed above were calculated using the methods of Deevey (1947) and Birch (1948). Some life tables were terminated before all individuals had died, but the contribution of those remaining to the vital statistics would have been minimal (Hall 1964). Occasionally, broods were retained in separate glasses and held through several molts for observation of any second-generation effects that might not be expected from the life table of the original cohort; none was observed.

RESULTS

Ankistrodesmus falcatus *(green alga)*

Under observation, both mounted and free *D. pulex* filled their guts with *A. falcatus* in less than 1 hr (Fig. 1). The estimated filtering rate was 2.2×10^6 cells/hr

TABLE 1. *Ingestion and assimilation of ^{14}C-labeled algae by groups of five* Daphnia pulex, *expressed as disintegrations per minute. All food counts adjusted to 5,116 dpm/50 ml at start of feeding. Food level K = 25*

Food material	Adjustment factor	Adjusted dpm of: 50 ml of labeled food at end of 1-hr feeding	food ingested (mean and SD of 5 groups)	food assimilated (mean and SD of 5 groups)	*t* test between ingestion and assimilation count means
Ankistrodesmus falcatus (green alga)	none	9,492	504 ± 57	358 ± 36	4.3486*
Anacystis nidulans	1.427	7,089	322 ± 61	51 ± 7	8.1182*
Synechococcus elongata	1.005	6,567	61 ± 4	41 ± 8	4.0491†
Merismopedia sp.	0.979	10,840	129 ± 22	110 ± 23	1.1910
Synechococcus cedrorum	2.608	9,519	262 ± 53	213 ± 70	1.1210
Anabaena flos-aquae	1.381	22,568	150 ± 43	213 ± 46	−2.0427
Synechocystis sp.	1.232	9,720	239 ± 31	186 ± 30	2.5156†
Gloeocapsa alpicola	0.868	9,369	309 ± 28	218 ± 13	5.9421*
Chlorella vulgaris (green alga)	1.139	9,620	262 ± 81	303 ± 28	−0.8819

* Significant at 99% confidence level.
† Significant at 95% confidence level.

(Fig. 2, Tables 1 and 2). The radiotracer experiments also showed rapid ingestion. The assimilation was only 71%, probably because feeding on this alga was very rapid, and food material was passed through the gut before there was time for it to be fully digested. The life table of a cohort fed on *A. falcatus* showed high survival and reproduction (Table 3, Figs. 3 and 4), agreeing with the reports of Hall (1964) and Frank et al. (1957), considering differences in species and techniques. Survival was never as good on any other food as on *A. falcatus*.

Chlorella vulgaris *(green alga)*

Ingestion of *C. vulgaris* by *D. pulex* was neither as high nor as consistent as of *A. falcatus* (probably because it settled out of suspension rapidly and was variable in its acceptance by *Daphnia*), so it was not used in the life-table experiments. Although *C. vulgaris* was ingested less than *Ankistrodesmus*, assimilation was 100% (Fig. 2, Tables 1 and 2). This further supports the idea that the difference in assimilation vs. ingestion of *A. falcatus* was due to the animals' inability to digest the large quan-

TABLE 2. *Estimated filtering rate (EFR) and percentages of assimilation (A) of green and blue-green algae by* Daphnia pulex *in ^{14}C tracer experiments at concentration K = 25. (Counts for available, ingested, and assimilated ^{14}C are in Table 1)*

Alga	Mean ^{14}C available (dpm)*	EFR† (10^6 cells hr^{-1} $individual^{-1}$)	A‡ (%)
Ankistrodesmus falcatus (green)	7,304	2.20	71.0
Chlorella vulgaris (green)	7,368	1.11	100§
Anacystis nidulans	6,102	1.65	15.8
Synechococcus elongata	5,841	0.33	67.2
Merismopedia sp.	7,978	0.51	85.3
Synechococcus cedrorum	7,317	1.12	81.3
Anabaena flos-aquae	13,843	0.34	100§
Synechocystis sp.	7,418	1.01	77.8
Gloeocapsa alpicola	7,242	1.33	70.6

* Available ^{14}C = ½ [dpm of 50 ml algae at start of feeding + dpm of 50 ml algae at end of feeding (1 hr)].

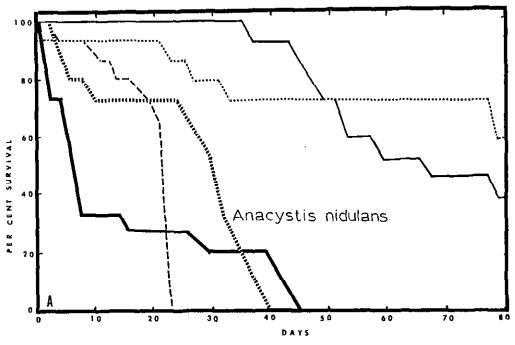
† Estimated filtering rate = [(156.25 × 10^6) (dpm of 5 *Daphnia*)] [5 (available ^{14}C dpm)]$^{-1}$. (100 ml at "K = 25" = 156.25 × 10^6 cells.)

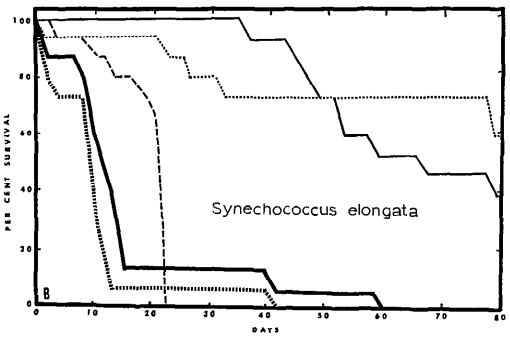
‡ Percent assimilation = (dpm ^{14}C assimilated) (dpm ^{14}C ingested)$^{-1}$ × 100.

§ Calculated percentage is greater than 100 but is taken as 100 since differences were not statistically significant.

TABLE 3. Daphnia pulex *life-table data for experimental and control foods*

Species (food)	Food level (K)	Net reproduction rate (R_0)	Age of death (days)		Rate of natural increase (r)
			Median	Range	
Ankistrodesmus falcatus (green alga)	25	2,906.5	66	35–>83	0.74
	1	306.9	>83	2–>83	0.37
Anacystis nidulans	25	2.1	6.5	2–46	0.09
	1	39.9	30.5	4–40	0.26
Synechococcus elongata	25	0.9	11.5	2–60	≪0.01
	1	0.1	9	2–42	≪0.01
Merismopedia sp.	25	0	5	2–15	0
	1	0	7.5	2–15	0
Synechococcus cedrorum	25	225.4	58	4–>73	0.43
	1	181.9	>73	8–>73	0.29
Anabaena flos-aquae	25	82.4	>32	18–>32	0.21
	1	34.1	>32	2–>32	0.16
Synechocystis sp.	25	0	17	2–24	0
	1	0	34	8–>60	0
Gloeocapsa alpicola	25	0	4	2–8	0
	1	0	14.5	6–>77	0
Yeast	10	1,289.2	52.5	2–85	0.57
No food (sterile water)	—	1.6	21.3	4–23	0.04


PER CENT SURVIVAL
DAYS
Anacystis nidulans
A


PER CENT SURVIVAL
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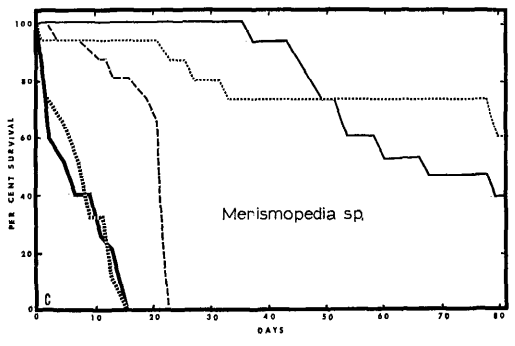
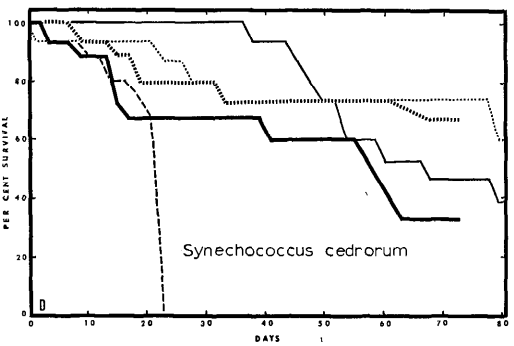

PER CENT SURVIVAL
DAYS
Merismopedia sp.
C


PER CENT SURVIVAL
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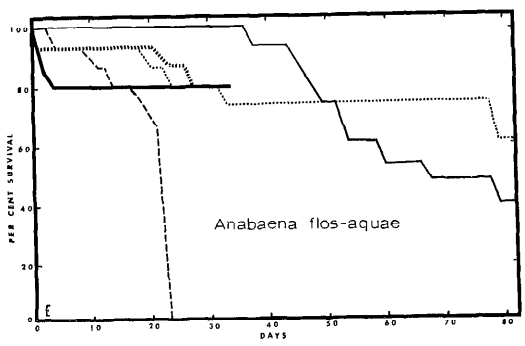
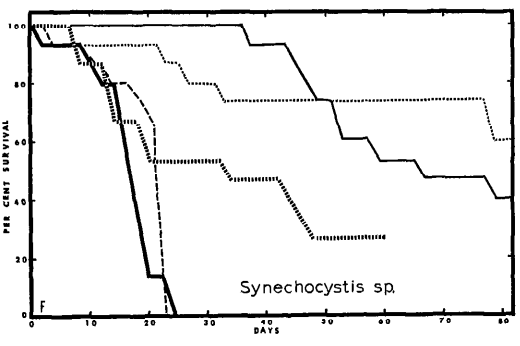
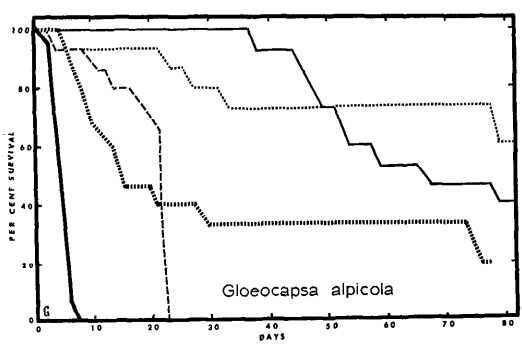
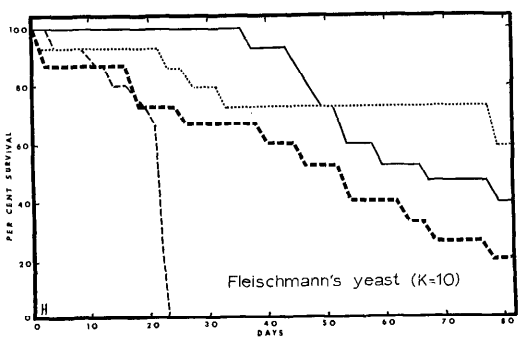
Synechococcus cedrorum
D


PER CENT SURVIVAL
DAYS
Anabaena flos-aquae
E


PER CENT SURVIVAL
DAYS
Synechocystis sp.
F


PER CENT SURVIVAL
DAYS
Gloeocapsa alpicola
G


PER CENT SURVIVAL
DAYS
Fleischmann's yeast (K=10)
H

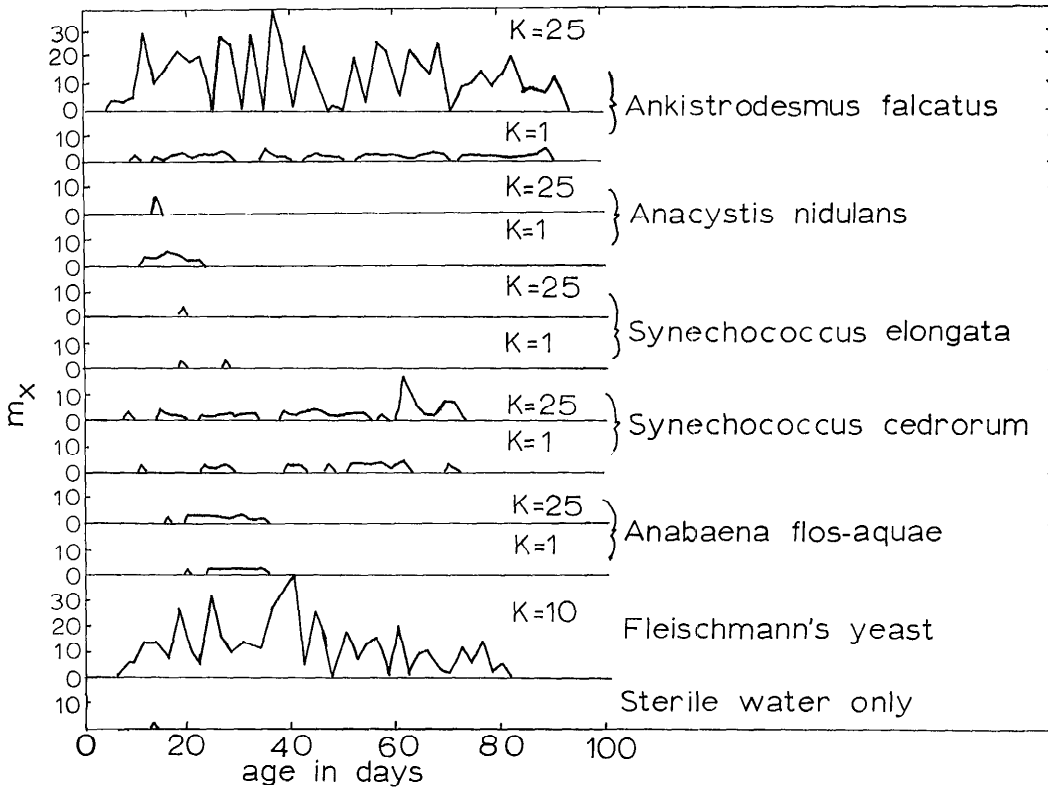


FIG. 4. Age-specific reproductive rate (m_x) of *Dapnia pulex* fed various food suspensions which supported reproduction.

tities of algae ingested before they were forced out of the gut. The lower rate (1.11 $\times$ 10^6 cells/hr) at which *C. vulgaris* was ingested (Fig. 1) probably allowed time for complete digestion and assimilation of the algae. Mean assimilation values for *C. vulgaris* (and *A. flos-aquae*) from the radiotracer experiments were actually higher than the mean values for ingestion, but the differences were not statistically significant (Table 1). Taub and Dollar (1968) found that *D. pulex* did not have normal longevity or reproduction when fed the closely related *C. pyrenoidosa*, but their definition of normal was rather obscure. My results did not support the finding of Smith (1937) that minute Chlorophyceae were eaten more readily by *Daphnia* than was *Ankistrodesmus*.

Chlamydomonas *sp. (green alga)*

Although *Chlamydomonas* sp. was readily accepted by *D. pulex* under observation (Fig. 1), it does not remain uniformly distributed in a suspension and was not used in radiotracer or life-table experiments. *Daphnia* filled their guts with *Chlamydomonas* sp. in less than 1 hr. Taub and Dollar (1968) found *Chlamydomonas reinhardi* inadequate for normal reproduction and longevity in *D. pulex*. Frank et al. (1957)

←

FIG. 3. Survivorship of *Daphnia pulex* (groups of 15 individuals) fed various food suspensions at two concentrations. Heavy solid line—experimental food, K = 25 concn; barred line—experimental food, K = 1 concn; dashed line—sterile water only; solid line—*Ankistrodesmus falcatus*, K = 25 concn; dotted line—*A. falcatus*, K = 1 concn.

fed *D. pulex* on *Chlamydomonas moewusi* and found survivorship and reproduction values considerably lower than those I found for *D. pulex* fed on *A. falcatus*. Schindler (1968) reported that assimilation rates of *Chlamydomonas* and *Chlorella* fed to *D. magna* were not significantly different.

Fleischmann's yeast

Yeast was used to provide a nonalgal comparison food, and was more or less intermediate in results between blue-greens and green algae. Although *D. pulex* fed on yeast suspensions, they seemed unable to do so efficiently; guts were only about two-thirds filled after 1 hr (Fig. 1). Fedorov and Sorokin (1967) found that yeasts were assimilated better than algae by *Daphnia longispina*. I did no radiotracer experiment with yeast, so I cannot confirm this for *D. pulex*. The life-table experiment (Figs. 3H and 4, Table 3) suggests that yeast is not as good a food as *A. falcatus* but is better than any of the blue-green algae tested. Reproduction was high and quite regular ($R_0 = 1{,}289.2$, $r = 0.57$). Yeast suspensions could only be used at density K = 10, since K = 25 caused oxygen depletion in the water.

Sterile water

Radiotracer experiments run with sterile water only (no algae) with and without ^{14}C added showed that the *D. pulex* had no radioactive contamination and that neither the *Daphnia* nor the Millipore filters picked up detectable radiation from solution. The life-table experiment with sterile water (Figs. 3 and 4, Table 3) showed higher longevity than expected (21.3 days median, 23 days maximum) but still very poor survivorship and reproduction ($r = 0.04$). Only two newborn were produced, both in one clutch. The energy for this production and longevity may have come from imperfectly cleaned glassware or other experimental error.

Anacystis nidulans

Anacystis nidulans was ingested only rarely by *D. pulex* under observation (Fig. 1). The filtering rate was 1.65×10^6 cells/hr, but assimilation was only 15.8% (Fig. 2, Tables 1 and 2). The life table (Figs. 3A and 4, Table 3) of *D. pulex* fed *A. nidulans* showed poor survival and reproduction ($R_0 = 2.1$ and 39.9, $r = 0.09$ and 0.26 at high and low food levels respectively). Survival and reproduction at the high food level were lower than on the low food level, indicating a possible inhibitory effect.

Synechococcus elongata

Daphnia pulex seldom ingested *S. elongata*. The estimated filtering rate was 0.33×10^6 cells/hr (Fig. 1). In the radiotracer experiments (Fig. 2, Tables 1 and 2) *S. elongata* showed only 67% assimilation; in this case both ingestion and assimilation were very low, suggesting some mechanism in the *Daphnia* for sensing and rejecting this alga. The life table for *D. pulex* fed *S. elongata* (Figs. 3B and 4, Table 3) showed a rapid decline in survivorship followed by extended longevity in a few individuals. This pattern was also noticed with some other blue-green algae and could be due to some sort of acclimatization process. Reproduction ($R_0 = 0.9$, $r = \leqslant 0.01$ on high food and 0.1, $\leqslant 0.01$ on low) and median longevity (11.5 and 9 days) were both very low.

Merismopedia *sp.*

Daphnia was not seen ingesting significant numbers of *Merismopedia* sp. (Fig. 1). The estimated filtering rate was 0.51×10^6 cells/hr. The radiotracer experiment, however, indicated some ingestion and nearly complete (85.3%) assimilation of ingested material (Fig. 2, Tables 1 and 2). Life-table data for *Daphnia* fed *Merismopedia* showed low survivorship (lower than the control group in sterile water) and no reproduction at either food level (Fig. 3C, Table 3). Taken together, these facts suggest that the particular strain used may have had toxic properties not previously reported for *Merismopedia*.

Synechococcus cedrorum

I did not observe *D. pulex* ingest *S. cedrorum*. The animals did acquire a green tinge

in the gut, indicating that some material from the cell was ingested (Fig. 1), and in the radiotracer experiments (Fig. 2, Tables 1 and 2), ingestion and assimilation were both quite high. Estimated filtering rate was 1.12×10^6 cells/hr, with 81.3% assimilation. Also, the life-table experiment showed the highest survivorship (median longevity 58 days, max >73, on high and low food) and reproduction ($R_0 = 225.4$ and 181.9, $r =$ 0.43 and 0.29) of any of the blue-greens (Figs. 3D and 4, Table 3). This is in sharp contrast to the congeneric *S. elongata* discussed above, but even different strains of the same algal species may vary greatly in nutritional value (Provasoli et al. 1959). Gassner (1962) found significant differences in pigment between *S. elongata* and *S. cedrorum*, which may be related to the nutritional differences found here.

Anabaena flos-aquae

Daphnia pulex was not seen taking in *A. flos-aquae*, but their guts acquired a slight green tinge, indicating some ingestion (Fig. 1). Estimated filtering rate was only 0.34×10^6 cells/hr, but assimilation was 100% (Fig. 2, Tables 1 and 2). *Daphnia* may break up the short filaments of this alga in the filtering process, rendering it less obvious in the gut. Life-table experiments with *A. flos-aquae* had to be terminated before completion, but there was high survival and some reproduction in the first 32 days (Figs. 3E and 4). R_0 values of 82.4 and 34.1 were calculated for the high and low food levels based on the data for 32 days. Values for r (0.21 and 0.16) were not affected by the early termination (Table 3). Although *A. flos-aquae* has been reported as toxic (Gorham 1965), the strain I tested was evidently not so to *D. pulex*. Schindler (1968) reported that *D. magna* assimilated *Anabaena* at a much lower rate than *Chlamydomonas* or *Chlorella*, and Sorokin (1968) stated that some species of *Anabaena* were not consumed at all by zooplankton.

Synechocystis *sp.*

Like the last two species, *Synechocystis* sp. appeared to be ingested only slightly by *D. pulex* (Fig. 1) but was ingested and assimilated in radiotracer experiments (Fig. 2, Tables 1 and 2). Estimated filtering rate was 1.01×10^6 cells/hr and assimilation was 77.8%. Survivorship at the high food level was very low, but at the low food level it was somewhat better. No reproduction occurred at either level (Fig. 3F, Table 3). These facts suggest that although *Synechocystis* may be ingested by *D. pulex*, it has little nutritional value and may be toxic in high concentrations.

Glococapsa alpicola

Only one of the six *D. pulex* observed actually ingested *G. alpicola* (Fig. 1), but all six acquired some color in the gut. The ingestion and assimilation of radioactive *G. alpicola* were both substantial, estimated filtering rate 1.33×10^6 cells/hr, with 70.6% assimilation (Fig. 2, Tables 1 and 2). Despite this high utilization, *G. alpicola* evidently had little nutritional value, as shown by the life-table experiment (Fig. 3G). Survival of *D. pulex* on the high level of *G. alpicola* was the poorest of any of the experiments and was low at the low food level (Table 3). No reproduction whatever occurred. The low survival in comparison to controls in sterile water may indicate a toxic or inhibiting effect.

DISCUSSION

It is well established that *Daphnia* can to some extent select the material that they filter from the water (*see* Burns 1969) and that they can utilize a variety of foods if necessary. Brooks and Dodson (1965) pointed out that large Cladocera such as *Daphnia* can collect particles in the 1–15 μ size range which includes a heterogeneous mixture of algae, bacteria, and organic detritus. The relative proportions of the components may change, but the mixture constitutes a relatively constant and adequate source of food.

My study implies that if the food mixture becomes dominated by blue-green algae, the Cladocera may survive only at a very low level of abundance. The genera commonly forming blooms in nature, including *Anabaena* and *Anacystis*, are normally quite

large, except when fragments or small groups of cells break away from the main thallus. The turbulent culture conditions that I used to keep large thalli from forming may represent conditions in nature only at certain times. The other species of blue-green algae that I used occur in the plankton as single cells or small groups of cells. The *Daphnia* were able to filter all of the tested foods from the medium without mechanical interference, and all foods were ingested to at least a slight degree, implying that rejection was based on some other factor. The most rejected species were all blue-greens. Their toxic extracellular products (Fogg 1962; Lefèvre 1964) may account for such rejection if they can be sensed by *D. pulex*.

In many of my experiments, survival of *D. pulex* fed on blue-greens was lower at the high (K = 25) food level than at the low (K = 1) level, suggesting inhibitory effects of the food rather than poor nutritional value. The data for *A. falcatus* are anomalous in this respect, with better survival at the high food level up to 50 days but considerably lower survival after that; this may be related to the far greater reproductive output of the high food group (R_0 = 2,906.5; r = 0.74 vs. R_0 = 306.9, r = 0.37). There is some disagreement as to the nutritional value of blue-green algae (e.g., *see* Schwimmer and Schwimmer 1955 vs. Bowman et al. 1962); probably, as in the case of green algae, protein levels vary depending on environmental conditions (Spoehr and Milner 1949; Taub and Dollar 1965). According to Blâzka (1966), *Daphnia hyalina* does not use proteins for metabolism in laboratory culture at surplus food levels and only in limited amounts in the field: nonprotein substrates are preferred and proteins are emergency food. If blue-greens are in general highly proteinaceous and if his findings hold for *D. pulex*, the low growth and reproduction in my experiments might be expected.

Copepods, rotifers, and some Cladocera may utilize blue-green algae to a greater degree than the *D. pulex* used here. Blâzka (1966) reported successful growth and reproduction of *D. pulicaria* feeding on a bloom of blue-green algae; it is possible that bacteria present were an important factor. In my experimental procedures, I attempted to minimize interference by bacteria as a source of supplemental food; at least control and experimental foods should have had similar bacterial contamination. Antibiotics were not used, since Provasoli et al. (1959) and others have found that such treatments are inhibitory to *Daphnia*. My procedures may thus have exposed the poor food value of the blue-green algae more than observations in nature.

Most workers who have used radioactive food to study *Daphnia* and other Crustacea have used dry counting methods (Sorokin 1968; Rigler 1971). This usually creates problems due to self-absorption of radiation by the animals. The liquid scintillation method offers several advantages over counting on planchets. Bell and Ward (1968) used liquid scintillation to determine self-absorption by *D. pulex*. Self-absorption in liquid scintillation counting may be reduced to negligible levels through the use of a tissue solubilizer, such as NCS, which dissolves the soft tissues of the animal; the hard carapace becomes transparent and is saturated with scintillation solution. Ward et al. (1970), also using *D. pulex*, showed that such a procedure sharply reduces self-absorption of light and beta radiation and eliminates the need for correction factors. The carapaces do settle to the bottom of the vial, but settling did not result in a loss of counting efficiency (Bell and Ward 1968).

My experiments were kept short to minimize any loss of assimilated ^{14}C by respiration or excretion. Schindler (1968) reported that *D. magna* filled their guts with radioactive food (in lower concentrations than those I used) in less than 1 hr but did not respire radioactive carbon dioxide until more than 16 hr after beginning to ingest the food. Monakov and Sorokin (1961) obtained a loss rate of only 14–16% per day for ^{14}C from *D. pulex*. Schindler (1968) also found that *D. magna* cleared their guts of radioactive food in from 30–60 min after transfer to nonradioactive food. My *D. pulex* were kept in radioactive food for 1 hr

and allowed 2 hr to clear their guts in nonradioactive food. This should have prevented errors due to incomplete filling of guts with nonradioactive food, excretion, and respiration.

REFERENCES

ANDERSON, B. G., H. LUMER, AND L. J. ZUPANCIC, JR. 1937. Growth and variability in *Daphnia pulex*. Biol. Bull. **73**: 444–463.

BANTA, A. M. 1939. Studies on the physiology, genetics, and evolution of some Cladocera. Carnegie Inst. Wash. Pap. 39. 285 p.

BEERSTECHER, E. 1952. The nutrition of crustacea. Vitamins Hormones **10**: 69–77.

BELL, R. K., AND F. J. WARD. 1968. Use of liquid scintillation counting for measuring self-absorption of carbon-14 in *Daphnia pulex*. J. Fish. Res. Bd. Can. **25**: 2505–2508.

BIRCH, L. C. 1948. The intrinsic rate of natural increase of an insect population. J. Anim. Ecol. **17**: 15–26.

BIRGE, E. A. 1898. Plankton studies on Lake Mendota. 2. Trans. Wis. Acad. Sci. **11**: 274–451.

BLÂZKA, P. 1966. Metabolism of natural and cultured populations of *Daphnia* related to secondary production. Int. Ver. Theor. Angew. Limnol. Verh. **16**: 380–385.

BOWMAN, R. O., J. B. MIDDLEBROOK, AND R. H. MCDANIEL. 1962. Nutritional value of algae for mice. Aerosp. Med. **33**: 589–593.

BRAGINSKII, L. P. 1955. On toxic blue-green algae investigations [in Russian]. Priroda **44**: 117.

BROOKS, J. L., AND S. I. DODSON. 1965. Predation, body size, and composition of plankton. Science **150**: 28–35.

BURNS, C. W. 1968. Direct observations of mechanisms regulating feeding behavior of *Daphnia* in lakewater. Int. Rev. Gesamten Hydrobiol. **53**: 83–100.

———. 1969. Particle size and sedimentation in the feeding behavior of two species of *Daphnia*. Limnol. Oceanogr. **14**: 392–402.

CAIN, R. D. 1967. Detergents in membrane filters. Science **155**: 195–196.

DEEVEY, E. S., JR. 1947. Life tables for natural populations of animals. Quart. Rev. Biol. **22**: 283–314.

DILLENBERG, H. O., AND M. K. DEHNEL. 1960. Toxic waterbloom in Saskatchewan, 1959. Can. Med. Ass. J. **83**: 1151–1154.

EDMONDSON, W. T. 1957. Trophic relations of the zooplankton. Trans. Amer. Microsc. Soc. **76**: 225–246.

———. 1965. Reproductive rate of planktonic rotifers as related to food and temperature in nature. Ecol. Monogr. **35**: 61–111.

FEDOROV, V. K., AND JU. I. SOROKIN. 1967. Determination of the assimilability of algae, yeasts, and bacteria for some representatives of the Cladocera. Dokl.—Biol. Sci. Sect. (English Transl.) **174**(4): 969–970.

FITZGERALD, G. P. 1964. The biotic relations within water blooms, p. 300–306. *In* D. F. Jackson [ed.], Algae and man. Plenum.

FOGG, G. E. 1962. Extracellular products, chapter 30. *In* R. A. Lewin [ed.], Physiology and biochemistry of algae. Academic.

FRANK, P. W., C. D. BOLL, AND R. W. KELLY. 1957. Vital statistics of laboratory cultures of *Daphnia pulex* de Geer as related to density. Physiol. Zool. **30**: 287–305.

GASSNER, E. B. 1962. On the pigment absorbing at 750 mμ occurring in some blue-green algae. Plant Physiol. **37**: 637–639.

GENTILE, J. H., AND T. E. MALONEY. 1969. Toxicity and environmental requirements of a strain of *Aphanizomenon flos-aquae* (L.) Ralfs. Can. J. Microbiol. **15**: 165–173.

GORHAM, P. R. 1965. Toxic waterblooms of blue-green algae, p. 37–43. *In* C. M. Tarzwell [ed.], Biological problems in water pollution. U.S. Public Health Serv. Publ. 999-WP-25.

———, J. MCLACHLAN, U. T. HAMMER, AND W. K. KIM. 1964. Isolation and culture of toxic strains of *Anabaena flos-aquae* (Lyngb.) de-Breb. Int. Ver. Theor. Angew. Limnol. Verh. **15**: 796–803.

HALL, D. J. 1964. An experimental approach to the dynamics of a natural population of *Daphnia galeata mendotae*. Ecology **45**: 94–112.

LEFÈVRE, M. 1942. L'utilisation des algues d'eau douce par les cladocères. Bull. Biol. Fr. Belg. **76**: 250–276.

———. 1950. *Aphanizomenon gracile* Lem. cyanophyte défavorable au zooplankton. Ann. Sta. Cent. Hydrobiol. Appl. **3**: 205–208.

———. 1964. Extracellular products of algae, p. 337–367. *In* D. F. Jackson [ed.], Algae and man. Plenum.

MCMAHON, J. W., AND F. H. RIGLER. 1963. Mechanisms regulating the feeding rate of *Daphnia magna* Straus. Can. J. Zool. **41**: 321–332.

MONAKOV, A. V., AND JU. I. SOROKIN. 1961. The quantitative data on the nutrition of *Daphnia* [in Russian]. Tr. Inst. Biol. Vodokhran., Akad. Nauk SSSR **4**(7): 251–261.

PENNAK, R. W. 1953. Fresh-water invertebrates of the United States. Ronald. 769 p.

PROVASOLI, L., K. SHIRAISHI, AND J. R. LANCE. 1959 Nutritional idiosyncracies of *Artemia* and *Tigriopus* in monoxenic culture. Ann. N.Y. Acad. Sci. **77**: 250–261.

RIGLER, F. H. 1971. Zooplankton, p. 228–256. *In* W. T. Edmondson and G. G. Winberg [eds.], Secondary productivity in fresh waters. IBP Handbook 17.

SCHINDLER, D. W. 1968. Feeding, assimilation, and respiration rates of *Daphnia magna* under various environmental conditions and their relation to production estimates. J. Anim. Ecol. **37**: 369–385.

SCHWIMMER, M., AND D. SCHWIMMER. 1955. The role of algae and plankton in medicine. Grune and Stratton. 85 p.

SHILO, M. 1967. Formation and mode of action of algal toxins. Bacteriol. Rev. **31**(3): 180–193.

SLOBODKIN, L. B. 1954. Population dynamics in *Daphnia obtusa* Kurz. Ecol. Monogr. **24**: 69–88.

SMIRNOV, N. N., AND O. I. FEOKLISTOVA. 1963. The toxicity of blue-green algae [in Russian]. Tr. Inst. Biol. Vodokhran., Akad. Nauk SSSR **5**(8): 10–12.

SMITH, M. W. 1937. Notes on the food of *Daphnia pulex* (deGeer) in fertilized water. Trans. Amer. Fish. Soc. **66**: 287–290.

SOROKIN, JU. I. 1968. The use of ^{14}C in the study of the nutrition of aquatic animals. Mitt. Int. Ver. Theor. Angew. Limnol. 16. 41 p.

———, A. V. MONAKOV, E. D. MORDUKHAI-BOLTOVSKAYA, E. A. TSIKHON-LUKANINA, AND R. A. RODOVA. 1965. Application of C^{14} method for studies of trophic role of blue-green algae, p. 235–239. *In* Ecology and physiology of blue-green algae [in Russian]. Akad. Nauk SSSR.

SPOEHR, H. A., AND H. W. MILNER. 1949. The chemical composition of *Chlorella*; effect of environmental conditions. Plant Physiol. **24**: 120–149.

STANGENBERG, M. 1968. Toxic effects of *Microcystis aeruginosa* Kg. extracts on *Daphnia longispina* O. F. Müller and *Eucypris virens* Jurine. Hydrobiologia **32**: 81–87.

STARR, R. C. 1964. The culture collection of algae at Indiana University. Amer. J. Bot. **51**: 1013–1044.

STEEL, R. G. D., AND J. H. TORRIE. 1960. Principles and procedures of statistics. McGraw-Hill. 481 p.

TAUB, F. B., AND A. M. DOLLAR. 1965. Control of protein level of algae, *Chlorella*. J. Food Sci. **30**: 359–364.

———, AND ———. 1968. The nutritional inadequacy of *Chlorella* and *Chlamydomonas* as food for *Daphnia pulex*. Limnol. Oceanogr. **13**: 607–617.

VANCE, B. D. 1965. Composition and succession of cyanophycean water blooms. J. Phycol. **1**: 81–86.

WANG, C. H., AND D. L. WILLIS. 1965. Radiotracer methodology in biological science. Prentice-Hall. 382 p.

WARD, F. J., B. WONG, AND G. G. C. ROBINSON. 1970. A liquid scintillation procedure for determining the effect of size on self-absorption of ^{14}C in *Daphnia pulex*. Limnol. Oceanogr. **15**: 648–652.