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SMALL SCALE MASS CULTURE OF DAPHNIA MAGNA STRAUS

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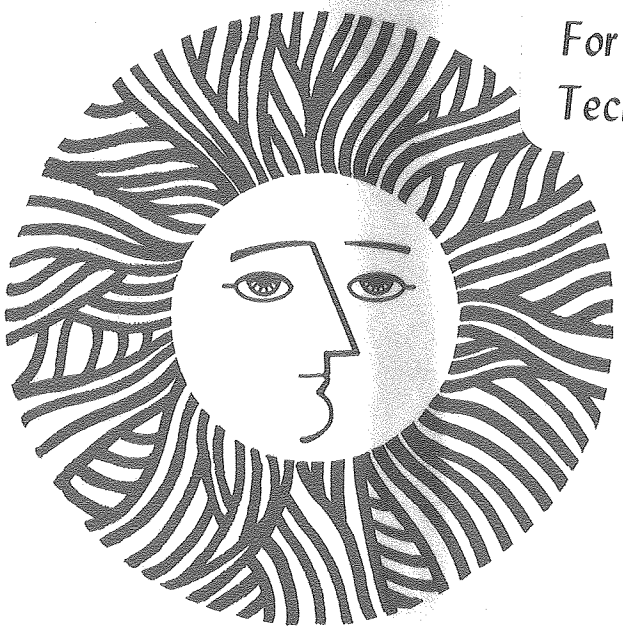

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ABSTRACT

Daphnia magna Straus 1820 was reared on a defined medium in 4-liter flasks under controlled conditions of light, temperature and species of algal food. Adult *D. magna* were found to be tolerant to high levels of ammonia, up to 108 μM, at high pH (>10), although parthenogenic reproduction may be inhibited at these high levels. *Scenedesmus quadricauda* and *Ankistrodesmus* sp. were found to be satisfactory food sources. Densities of greater than one animal per ml in culture were attained utilizing *Ankistrodesmus* sp. as a food source at a pH of 7.7. Maintenance of pH at around 7-8 appears to be important to successful *D. magna* culture.

INTRODUCTION

General cultivation methods for the laboratory rearing of fresh water cladocerans of the family Daphniidae have evolved in sophistication from the "stable tea" of Banta (1939) to the defined media of Murphy (1970) and D'Agostino and Provasoli (1970). Interest in the laboratory cultivation of the Daphniidae has centered around topics such as animal population dynamics (Slobodkin, 1954) and fresh water trophic levels (Taub and Dollar, 1968). The study of mass culture of members of the Daphniidae and assessing its potential application as a food source in fresh water aquaculture has not, to the authors's knowledge, been pursued.

The brine shrimp Artemia has been the food source of choice in aquacultural work on many levels due to its relatively low cost, accessibility, and ease of handling with regard to hatching of the nauplii from resting eggs, which, if properly stored, remain viable for years. All carnivorous larval and adult aquatic forms of a suitable size to eat them seem to relish Artemia. Artemia cysts are transported all over the world, and when a supply of live food is needed, Artemia eggs need only be added to a suitable hatching medium. Within 48 hours food is available. Newly hatched and adult Artemia are used in a wide variety of aquacultural endeavors from commercial Macrobrachium culture to experimental lobster culture. Alternatives and supplements to an Artemia diet in aquaculture might be welcomed for a number of reasons, but most persuasive is a food source which is not subject to disruptions in supply. As the world-wide transportation system becomes more vulnerable to petroleum shortages and increasing costs, a ready supply of live food may become more attractive.

Daphnia magna was chosen for experimental work in the present study because, as the largest known fresh water cladoceran, it offered the widest range of potential sizes as a food source. Daphnia and related genera have admirable attributes for their potential use in aquaculture, such as ready availability, nutritional acceptability and above all, parthenogenic reproduction, which allows for large population accrual in a relatively short period of time.

METHODS AND MATERIALS

Preliminary Experiments

A number of trial runs in one-liter, stoppered, aerated flasks were undertaken to ascertain which medium or combination of media provided the best overall growth of Daphnia magna. Two "synthetic" algal media, 2 "natural" Daphnia media, 2 "synthetic" Daphnia media, and 4 species of algae were tried in various combinations in promoting growth and reproduction in Daphnia magna (Table 1). General algal media consist of various mineral salts with or without vitamin supplements, while general Daphnia media consist of an organic enrichment and/or a vitamin supplement. One aim of the study was to use as inexpensive and easily accessible methods wherever possible. Local algae was isolated as a food source for the Daphnia by methods outlined by Hoshaw and Rosowski (1973), although axenic methods involving antibiotics were avoided. Efficacy of media and algal species was assessed by qualitative observations on D. magna growth, reproductive rate, and behavior. Successful combinations were used in the Primary Experiments.

Primary Experiments

Two primary experiments, designated Runs I and II, were established, consisting of 5 4-liter Ehrlenmeyer flasks each. The flasks were set up under a bank of fluorescent lights which received approximately 8750 lux of illumination on a 12 hr:12 hr light-dark cycle. The flasks were stoppered, and gentle aeration was provided with Silent Giant pumps and glass tubing inserted into the flasks. A 1 μ millepore filter was inserted into the aeration line between the pump and the flasks to prevent contamination. Ambient temperature was controlled at 19±1°C. The flasks were washed and sterilized prior to being filled with media.

In Run I each of the flasks were initiated with the following: (1) 3640 mls of Taub's media 63, (Taub and Dollar, 1968) combined with Murphys's medium (Murphy, 1970), the latter minus calcium acetate, penicillin, streptomycin, and bovine serum albumin, (2) 1000 mls filtered, autoclaved tank water from a laboratory aquarium stocked with guppies and aquatic plants, and (3) 60 mls of soil extract (see Nichols, 1973). Day 1 was established at the time when the media were mixed. Twenty-five mls of a pure, non-axenic laboratory-grown culture of Scenedesmus quadricauda (UTEX 76) at a concentration of 1.6×10^5 cells ml^{-1} were added to flasks 1 through 4 on day 1, and an additional 100 mls of S. quadricuada at the same concentration was added on day 6. Three Daphnia magna about 2.5 mm in length were added to flasks 1, 2, and 3 on day 4. The final experimental set-up consisted of 3 flasks with media, algae, and D. magna (flasks 1, 2, and 3), one flask with media and algae only (flask 4), and one flask with media only (flask 5). To prevent contamination of flasks 1-3 all Daphnia were rinsed with distilled water on a Nitex mesh and then placed in Taub's media without any algal food. After 24 hrs they were placed in Taub's media with Scenedesmus in order to cleanse the gut of any potential contaminating algae. At weekly intervals beginning on day 5

the following parameters were measured: pH, with an Orion 601 electrode pH meter; NH_4, determined by the blue indophenol reaction and the absorbance measured with a Zeiss PM2 DL spectrophotometer (Solorzano, 1969); nitrate plus nitrite (NO_3 + NO_2), determined by reduction and diazotization and measured with the spectrophotometer (Golterman, 1969); phytoplankton numbers counted in a Sedgwich-Rafter cell under a Reichert Zetopan phase contrast microscope; Daphnia magna numbers, counted by eye, or by taking a subsample and extrapolating to an estimated number. Bacteria numbers were estimated by plate counting (APHA, 1971). In order to minimize the possibility of contamination by other species of algae and ciliates, one sample for all measurements was taken out of each flask at one time by means of inserting on autoclaved glass tube and withdrawing a 25 ml sample.

Run II was initiated, maintained, and measured in the same manner as Run I with the following changes and additions: (1) one ml per liter of .44 M solution of $NaHCO_3$ was added to the flasks to increase the buffering capacity of the system; (2) Ankistrodesmus sp., added from non-axenic cultures isolated from a local lake phytoplankton, was utilized as an algal food scource; (3) 4 ml each of .6 M $NaNO_3$ and .1 M K_2HPO_4 were added to flasks 1, 2, and 3 on day 14 to promote the growth of Ankistrodesmus; and (4) 50 mls of Ankistrodesmus at a concentration of 1.6×10^3 cells ml^{-1} was added to flasks 1, 2 and 3 on day 21 to replenish algae grazed by the Daphnia.

RESULTS

Preliminary Experiments

Table 2 gives the major results obtained in the preliminary 1-liter flasks. The best and most reliable Daphnia magna growth and

reproduction occurred in a Taub's + Murphy's media with soil extract, with the algae Scenedesmus or Ankistrodesmus as food. Adding bovine serum albumin seemed to stimulate growth, but only if added after algal and Daphnia populations had been established in the flasks. If added initially to the cultures, bacterial growth overwhelmed the systems. Chlorella pyrenoidosa (UTEX 251) and Chlamydomonas reinhardii (UTEX 89), used as algal food for cladocerans in previous work (Taub and Dollar, 1968; Murphy, 1970; D'Agostino and Provasoli, 1970), were generally unsuccessful. On the basis of these results, Scenedesmus quadricauda and Ankistrodesmus sp. were used as a food source for the Daphnia in the primary experiments.

Primary Experiments

The interrelationships among pH, Daphnia magna numbers and ammonia concentration is illustrated in Figure 1 (a-f). In Run I, in which measurements were taken for 42 days, and in which Scendesmus quadricauda was used as a food source for the Daphnia, the pH of all systems except 5 (that flask without algae) showed sudden and dramatic increases such that values reached between 10.0 and 10.3 by day 14. Throughout Run I flask 5 maintained a fairly stable pH between 7.4 and 7.9. Numbers of D. magna in flasks 1, 2, and 3 increased to maximum numbers of 20-28 per liter by day 20, and then declined thereafter, so that by day 42 there were only about 4 to 16 per liter (Fig. 1b). Ammonia levels varied among the flasks (Fig. 1c). Values in flasks 1 thorugh 4 rose to maximum levels by day 20 (44-107 μM), with flasks 1-3, those flasks containing Daphnia, displaying the highest levels (74-107 μM). Ammonia levels in flask 5 (nutrients only) rose steadily and attained a maximum value of 232 μM by the last day of the run. All flasks in both runs were

found to harbor bacteria by day 7, although the source of the bacterial contamination in the number 5 flasks is unknown. The 1μ filter in the air line was effective in screening algal and protozoan spores, but may not have been effective for filtering bacteria. The high ammonia levels in both runs in the number 5 flasks was undoubtedly due to the presence of bacteria.

In Run II, in which Ankistrodesmus was employed as a food source, pH levels in all flasks displayed the same pattern of change as in Run I up to day 14, i.e., increased levels of pH for flasks 1-4 to over 10.5 (Fig. 1d). Beyond day 14, flasks 1-3, which had initial identical innoculation procedures, began to vary in pH levels, such that by day 22, the last day of Run II, the pH of flask 2 had dropped to about 8.2, while flasks 1 and 3 showed values of 10.3 and 10.8 respectively. As in Run I, the pH of flask 5, that flask with nutrients only, showed a fairly stable pH which, after day 6, varied between about 7.8 and 8.0. Daphnia numbers increased dramatically in flasks 1 and 2, and by day 26 had increased to levels of 500 per liter (Fig. 1e). Numbers in flask 3 never exceeded 30 per liter and by day 33 all had died. Ammonia concentration showed a similar behavior with Run I, with flask 5 achieving the highest value by day 26 (95 μM), and flasks 1-3 showing intermediate increases (from 57 - 77 μM) respectively (Fig. 1f). By day 33 Flask 4, which had algae and nutrients but no D. magna, showed the slightest ammonia increase, and achieved a peak value of 16 μM on day 20.

DISCUSSION

The data suggests that there is a direct relationship between the pH and the numbers of Daphnia magna which ultimately appeared in the flasks. In Run I the pH of those flasks into which algae were introduced (1 through 4) never fell below 10 after day 14. The Daphnia in flasks 1-3 in Run I, which had reproduced up to day 20, did not do so after this time. In flask 2 of Run II, in which the pH dropped to 8.2, Daphnia numbers increased dramatically. A similar increase in Daphnia in flask 1 of Run II was not correlated with such a dramatic pH drop, although the pH did dip to 9.9 on day 26, rising again to 10.3 by day 33. In flask 3, which ultimately attained a pH high of 10.8, all Daphnia had died by day 33. Additionally, in 2 separate instances in which very high densities of Daphnia magna were attained under routine through non-rigorous culture conditions, low pH values were recorded (Table 3).

The relationship between the general health of an aquatic system and its pH has been well documented. Rises in pH in natural and laboratory systems are often correlated with photosynthetic activity, as removal of CO_2 by phytoplankton results in the decreased buffering capacity of the system (Wetzel, 1975, pp. 178-179). The rises in pH in the first 14 days in flasks 1 through 4 was undoubtedly due to high algal counts and correspondingly high photosynthetic activity. In Run I the Daphnia appeared unable to eat the Scenedesmus after day 14 as evidenced by lack of food in their guts and lack of parthenogenic reproduction, and algal counts remained high throughout the run (1×10^4 - 1×10^5 cells ml^{-1}). Ankistrodesmus cell counts in Run II fell to between $2.8 - 4.8 \times 10^2$ cells ml^{-1} for flasks 1, 2, and 3, which may have resulted in the lower

pH values recorded in Run II. The reason for the drop in pH in flasks 1 and 2 remains undetermined, although it may have been due to the ability of the Daphnia to eat the Ankistrodesmus despite the high pH, which was apparently not the case with Scenedesmus. Some species of algae may be better suited as a food source than others in this regard. Feeding rates for Daphnia pulex have been shown to fall to almost zero at pH values above 10 (Kring and O'Brien, 1976). Enhanced ammonia toxicity at high pH values has been documented in rainbow trout (Lloyd, 1961). Flasks that contained Daphnia attained ammonia concentrations of up to 107 µM, but levels of ammonia toxicity in the cladocera have not been determined. Nitrate + nitrite concentrations, NO_2 being another potential source of toxic effects, reached fairly high levels in both runs (up to 208 µM in flask 1 of Run I), but difficulties with the assay technique rendered our results questionable. Optimal pH in mass culture of Daphnia magna is probably around 7.0, as has been found for a wide variety of aquatic species under cultivation (Ackfors and Rosén, 1979). Daphnia pulex, however, has been shown to be able to adapt well to pH values as low as 6.5 (Kring and O'Brien, ibid). Further experiments are planned in which the pH of our systems will be artificially controlled.

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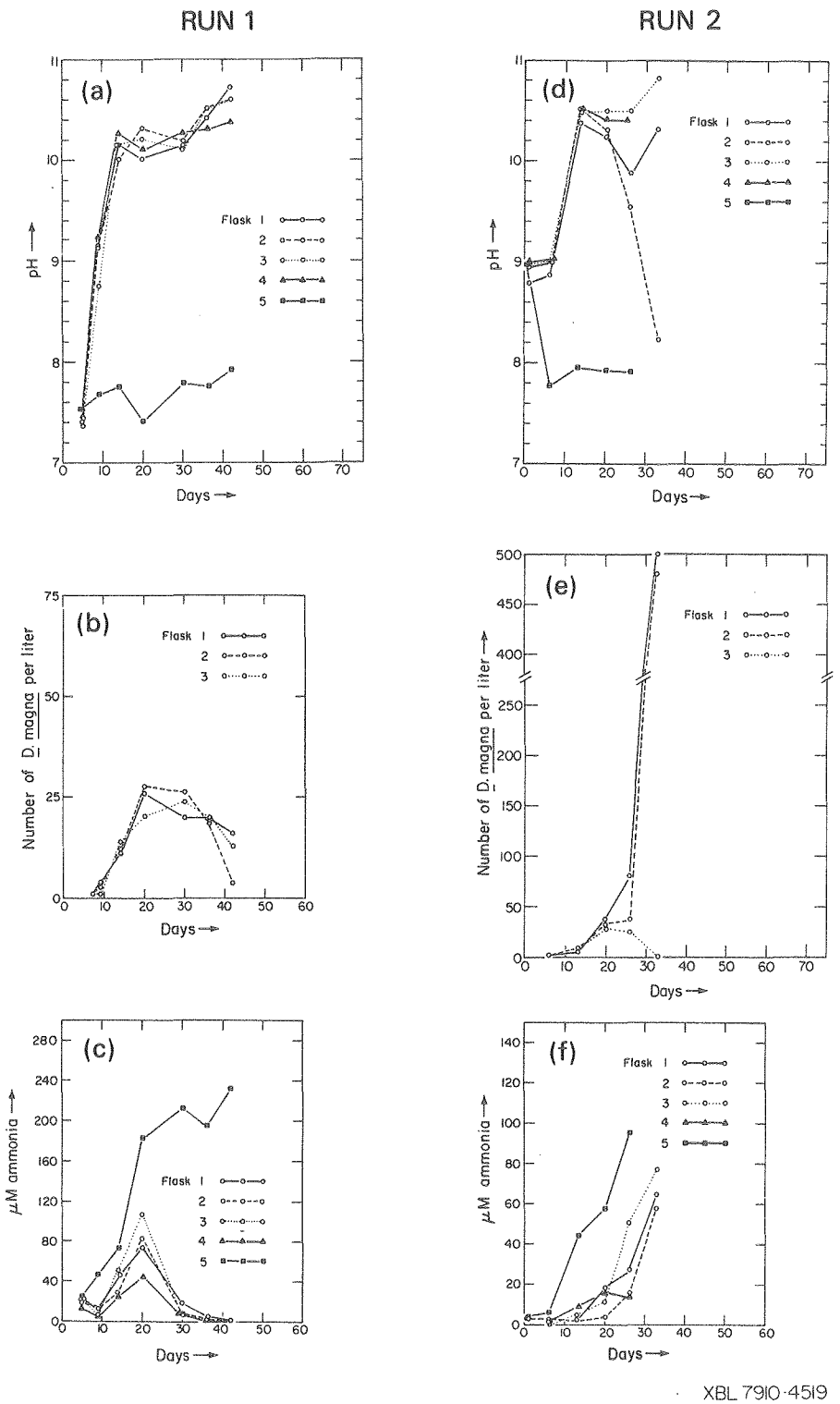

Figure 1. Comparison of pH, Daphnia magna numbers, and ammonia concentration over time in Runs I and II.

Table 1. Algal and Daphnia media used in preliminary experiments.

I. Synthetic algal media

a. Bold's Basal Medium (BBM; Nichols, 1973)

b. Taubs' media 63 (Taub and Dollar, 1968)

one ml per liter of .44 M Na HCO_3 was added as a buffer

II. Natural Daphnia media

a. Soil Extract — This is a sterile "tea" made by steaming, just below the boiling point, soil rich in organic matter in distilled water. (Nichols, 1973, p. 22)

b. Filtered, autoclaved tank water. — Any water in which aquatic organisms have lived for any length of time may be used. We used aquarium water in which guppies and aquatic plants had been well established for several months, and water from a large 200 liter container in our cold room stocked with cladocerans, copepods and ostracods and covered by a rich growth of duckweed (Lemna).

III. Synthetic Daphnia media

a. DM_2 + DA of D'Agostino and Provasoli (1970, Table I)

The phosphorus and nitrogen sources were omitted from our runs because we combined algal and Daphnia media in the same flask.

b. Murphy's medium (Murphy, 1970; Table I)

We omitted calcium acetate, penicillin, and streptomycin.

Table 2. Synopsis of one-liter flask experiments

Key:
+ + Excellent growth and reproduction
+ Some growth and reproduction
- no or little growth and reproduction

Experiment Number	Algal Medium	Daphnia Medium	Soil Extract (mls)	Filtered Autoclaved Tank Water (mls)	Algal Species	Duration of Experiment	Number of Flasks	Notes	Daphnia Growth
1	BBM		15		Chlamydomonas		1	irregular swimming behavior	-
2	BBM		15		Chlorella		1	irregular swimming behavior	-
3	BBM		7.5	500	Chlorella	21 days	1	Bacteria added but probably not needed	+ +
4	BBM		7.5	500	Chlorella	21 days	1	No bacteria added; results about the same	+ +
5	BBM		7.5	500	Chlamydomonas	21 days	2		-
6	BBM		7.5	500	Chlorella + Chlamydomonas	21 days	2	Slightly better than one above	+
7	BBM		7.5	500	Scenedesmus	10 days	1		+ +
8	Taub's 63				Scenedesmus	3 days	1		-
9	Taub's 63	thiotone only				1 day	1	cloudy culture water	-
10	Taub's 63	albumin only				1 day	1	cloudy culture water	-
11	Taub's 63	Murphy's	15				1		
12	Taub's 63	Murphy's	15	350	Chlorella	10 days	1		+
13	Taub's 63	Murphy's	15	500	Scenedesmus	25 days	3		+ +
14	Taub's 63	Murphy's		500	Scenedesmus	25 days	3		-
15	Taub's 63	Murphy's			Scenedesmus	13 days	1		-
16	Taub's 63	Murphy's	15		Scenedesmus	13 days	1		+
17	Taub's 63	Murphy's		500 (not autoclaved)	Scenedesmus	13 days	1		-
18	Taub's 63	Murphy's			Chlamydomonas + Scenedesmus		1		-
19	Taub's 63	Murphy's			Scenedesmus + Chlorella		1		-
20	Taub's 63	Murphy's	15	200	Scenedesmus	27 days	2		+
21	Taub's 63	Murphy's	15	200	Chlamydomonas	18 days	2		-
22	Taub's 63	Murphy's	15		Ankistrodesmus	17 days	1		+ +
23	Taub's 63	Murphy's	15		Scendesmus + Ankistrodesmus	17 days	1		+ +
24	Taub's 63	Murphy's			Scendesmus	18 days	2	Bovine albumin added on day 5	+ +
25	Taub's 63	Murphy's	15	200	Chlorella	18 days	2	Healthy Daphnia, but not as many as in Ankistrodesmus	+
26	Taub's 63	D'Agostino's + Provasoli's			Scenedesmus	18 days	2	Yeast extract & thiotone added on day 5; fewer Daphnia than with albumin; algae greener than with albumin	+
27	Taub's 63	Murphy's	15		Scenedesmus	15 days	2	fewer than with Ankistrodesmus	+
28	Taub's 63	Murphy's	15		Ankistrodesmus	15 days	2		+
29	Taub's 63	Murphy's	15		Scenedesmus	15 days	1	more Daphnia than with Ankistrodesmus; $NaHCO_3$ added on day 3	+
30	Taub's 63	Murphy's	15		Ankistrodesmus	15 days	1	$NaHCO_3$ added on day 3; algae growth poor	
31	Taub's 63	Murphy's	15		Scenedesmus	15 days	1	fewer Daphnia than with Ankistrodesmus; $NaHCO_3$ added at start	+
32	Taub's 63	Murphy's	15		Ankistrodesmus	15 days	1	$NaHCO_3$ added at start	+ +

Table 3. Parameters measured on a selected rearing beakers in which high *Daphnia magna* densities were acheived.

	Beaker 1	Beaker 2
size of beaker	one-liter	4-liter
age of culture	14 days	not known
species of algae food	axenic culture *Ankistrodesmus*	not known
growth media	Taub's + Murphy's + soil extract	not known
pH	7.65	7.30
ammonia concentration	4.7 µM	45.1 µM
Daphnia magna concentration (all sizes)	1100 liter^{-1}	830 liter^{-1}