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SMALL SCALE MASS CULTURE OF DAPHNIA MAGNA STRAUS

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ABSTRACT

We raised Daphnia magna Straus 1820 on a defined medium in 4-liter flasks with controlled light intensity, temperature, and algal food species. Adult D. magna tolerated high levels of ammonia (up to 108 μM) at high pH (> 10), although at these levels parthenogenic reproduction may be inhibited. Scenedesmus quadricauda and Ankistrodesmus sp. were satisfactory food sources, and by utilizing Ankistrodesmus we achieved densities greater than one animal per ml. Maintaining the pH at about 7-8 seems to be important for successful D. magna culture.

INTRODUCTION

General laboratory methods for cultivating freshwater cladocerans of the family Daphniidae have evolved from the "stable tea" of Banta (1939) to the defined media of Murphy (1970) and D'Agostino and Provasoli (1970). Laboratory cultivation of the Daphniidae has been utilized for studies on subjects such as animal population dynamics (Slobodkin, 1954) and freshwater trophic levels interactions (Taub and Dollar, 1968). To date, such studies have not attempted the mass culture of the Daphniidae as an assessment of their food source potential in freshwater aquaculture.

The brine shrimp Artemia has been the choice food in aquaculture work on many levels due to the relatively low cost, accessibility, and ease in hatching nauplii from resting eggs, which, if properly stored, remain viable for years. Within 48 hours after Artemia eggs are added to a suitable hatching medium, live food is available. All carnivorous larval and adult aquatic forms of a size suitable for eating Artemia seem to relish them; therefore, a variety of aquaculture systems use newly hatched and adult Artemia as a food. As international transportation becomes more vulnerable to petroleum shortages and cost increases, an alternative live food supply, not subject to interruptions, becomes increasingly important. For this reason and for reasons listed below, alternatives and supplements to the Artemia diet will be useful for future aquaculture work.

We studied the Daphnia magna because, as the largest known freshwater cladoceran, it offers the widest potential range of food sizes. In addition, D. magna and related genera are readily available, are nutritionally acceptable, and, most importantly, have parthenogenic reproduction, which accrues large populations in a relatively short time.

METHODS AND MATERIALS

Preliminary Experiments

We made a number of trial runs in one-liter, stoppered, aerated flasks, determining which medium or combination of media provides the best overall growth of Daphnia magna. Using inexpensive and easily accessible methods whenever possible, we selected two synthetic algal media, two natural cladoceran media, two synthetic cladoceran media, and four species of algae, in various combinations, to study D. magna growth (Table 1). General algal media consist of various mineral salts with or without vitamin supplements, while general cladoceran media consist of an organic enrichment and/or a vitamin supplement. Local algae were isolated as a food source for the D. magna by methods outlined by Hoslaw and Rosowski (1973), although axenic methods involving antibiotics were avoided. We assessed efficacy of media algal species 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 Run I and Run II, each used five 4-liter Ehrlenmeyer flasks. A bank of fluorescent lights over these flasks provided approximately 8,750 lux of illumination on a 12 hour: 12 hour light-dark cycle. Silent Giant pumps with glass tubing inserted into the stoppered flasks provide gentle aeration. To prevent algal contamination we inserted a 1 μ millepore filter into the aeration line between the pump and the flasks. The flasks were washed and sterilized prior to being filled with media, and the ambient temperature was controlled at $19 \pm 1^{o}C$.

To initiate Run I we filled each of the flasks with the following: 1) 3,640 mls of Taub's media 63 (Taub and Dollar, 1968) combined with Murphy'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 (Nichols, 1973). We measured day 1 from the time the media was 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 per ml were added to flasks 1-4 on day 1, and an additional 100 mls of S. quadricauda at the same concentration were 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 experiment used 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). We rinsed all D. magna with distilled water on a Nitex mesh and then placed them in Taub's media without algal food; subsequently, after 24 hours, they were placed in Taub's media with Scenedesmus in order to cleanse their guts of any potential contaminating algae.

Beginning on day 5 we measured the following parameters at weekly intervals: pH by an Orion 601 electrode pH meter; NH_4 by the blue indophenol reaction, with absorbance measured by a Zeiss PM2 DL spectrophotometer (Solorzano, 1969); nitrate plus nitrite ($NO_3 + NO_2$) by reduction and diazotization, with absorbance measured with the spectophrophotometer (Golterman, 1969); phytoplankton numbers by counting in a Sedgwich-Rafter cell under a Reichert Zetopan phase contrast microscope; Daphnia magna numbers by counting by eye, or by taking a subsample and extrapolating to an estimated number. We estimated bacteria numbers by plate counting (APHA, 1971). To minimize the possibility of contamination by algae and ciliates, we took at one time one sample for all measurements out of each flask by inserting an 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 0.44 M solution of $NaHCO_3$ was added to the flasks to increase the system's buffering capacity; 2) Ankistrodesmus sp., added from non-axenic cultures isolated from a local lake phytoplankton, was used as an algal food source; 3) 4 ml each of 0.6 M $NaNO_3$ and 0.1 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 per ml were added to flasks 1,2, and 3 on day 21 to replenish algae grazed by the Daphnia magna.

RESULTS

Preliminary Experiments

Table 2 shows major results from the preliminary experiments. The best and most reliable Daphnia magna growth and reproduction occurred in a Taub's plus Murpny's media with soil extract, using the algae Scenedesmus or Ankistrodesmus as food. Adding bovine serum to the algae seemed to stimulate growth, but only if added after algal and D. magna 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 either unpredictable or unsuccessful. Based on these results, we used Scenedesmus quadricauda and Ankistrodesmus sp. as food for D. magna in the primary experiments.

Primary Experiments

Figure 1 (a-f) shows the relationship between pH, Daphnia magna numbers, and ammonia concentration. In Run I, in which we took measurements for 42 days and used Scenedesmus quadricauda as a food source, the pH of all systems except in flask 5 (the flask without the algae) showed sudden and dramatic increases (pH of 10.0 - 10.3 by day 14). Throughout Run I flask 5 maintained a fairly stable pH of 7.4 - 7.9. Numbers of D. magna in flasks 1,2, and 3 increased to a maximum of 20 - 28 per liter by day 20 and then declined thereafter, so that by day 42 there were only about 4 - 16 per liter (Fig. 1b).

Ammonia levels varied among the flasks (Fig. 1c). Values in flasks 1 - 4 rose to maximum levels by day 20 (44 - 107 μM), with flasks 1 - 3 (those flasks containing D. magna) 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 harbored bacteria by day 7, although the source of the bacterial contamination in flask 5 is unknown. The 1 μ millepore filter in the air line effectively screened algal and protozoan spores but may not have been an effective filter for bacteria.

Bacteria presence undoubtedly caused the high ammonia levels in flask 5 in both runs.

In Run II with Ankistrodesmus 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., in flasks 1 - 4 pH levels increasing to over 10.5 (Fig. 1d). Beyond day 14 the pH levels in flasks 1 - 3, which had initial identical innoculation procedures, began to vary so that by day 22, the last day of Run II, the pH of flask 2 had dropped to about 8.2, while the pH in flasks 1 and 3 were 10.3 and 10.8 respectively. Similar to Run I, flask 5, with nutrients only, showed a fairly stable pH which varied after day 6 between about 7.8 and 8.0. Daphnia magna numbers increased dramatically in flasks 1 and 2 and by day 26 had increased to levels of 500 per liter (Fig. 1e). In flask 3 numbers never exceeded 30 per liter, and by day 33 all had died.

Ammonia concentration behaved in similar fashion to Run I. Flask 5 had the highest value by day 26 (95 μM), and flasks 1 - 3 showed intermediate increases (from 57 - 77 μM respectively - See Fig. 1f). By day 33 flask 4, which had algae and nutrients but no D. magna, showed the slightest ammonia increase, achieving a peak value of 16 μM on day 20.

DISCUSSION

The data suggest direct relationship between pH and the number of Daphnia magna ultimately appearing in the flasks. In Run I the pH in flasks 1 - 4 into which algae were added, never fell below 10 after day 14. For Run I in flasks 1 - 3 the D. magna, which had been reproducing, did not do so after day 20. D. magna increased dramatically in flask 2 for Run II, in which the pH dropped to 8.2. A similar increase in D. magna in flask 1 for Run II did not correlate 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 had a pH high of 10.8, all D. magna died by day 33. In addition, in two separate instances in which very high densities of D. magna were attained under routine but nonrigorous culture conditions, we recorded low pH values (Table 3).

The relationship between the general health of an aquatic system and its pH is well documented. Rises in pH in natural and laboratory

systems often correlate with photosynthetic activity as removal of CO_2 by phytoplankton results in decreased buffering capacity of the system (Wetzel, 1975, pp. 178 - 179). High algal counts and correspondingly high photosynthetic activity undoubtedly caused the rises in pH in flasks 1 - 4 during the first 14 days. In Run I the Daphnia magna appeared unable to eat the Scenedesmus after day 14 as shown by lack of food in their guts and lack of parthenogenic reproduction. Algal counts remained high throughout this run ($1 \times 10^4 - 1 \times 10^5$ cells per ml). Ankistrodesmus cell counts in Run II fell to $2.8 - 4.8 \times 10^2$ cells per ml for flasks 1,2, and 3, which may have resulted in the lower pH values of Run II. We do not know the reason for the pH drop in flasks 1 and 2, although D. magna eating the Ankistrodesmus, despite the high pH, may cause this drop. This apparantly was not the case with Scenedesmus; therefore, some species of algae may be better suited as a food source than others. Feeding rates for Daphnia pulex has been observed to drop dramatically 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 containing D. magna attained ammonia concentrations up to 107 µM. but levels of ammonia toxicity in cladocera are not known. Nitrate plus 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 for Run I), but difficulties with the assay technique rendered our results questionable. Optimal pH for mass culture of D. magna is probably around 7.0 as has been found for a wide variety of aquatic species under cultivation (Ackfors and Rosen, 1979). D. pulex, however, have been found to adapt to pH values as low as 6.5 (Kring and O'Brien, 1976). We plan additional experiments 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.

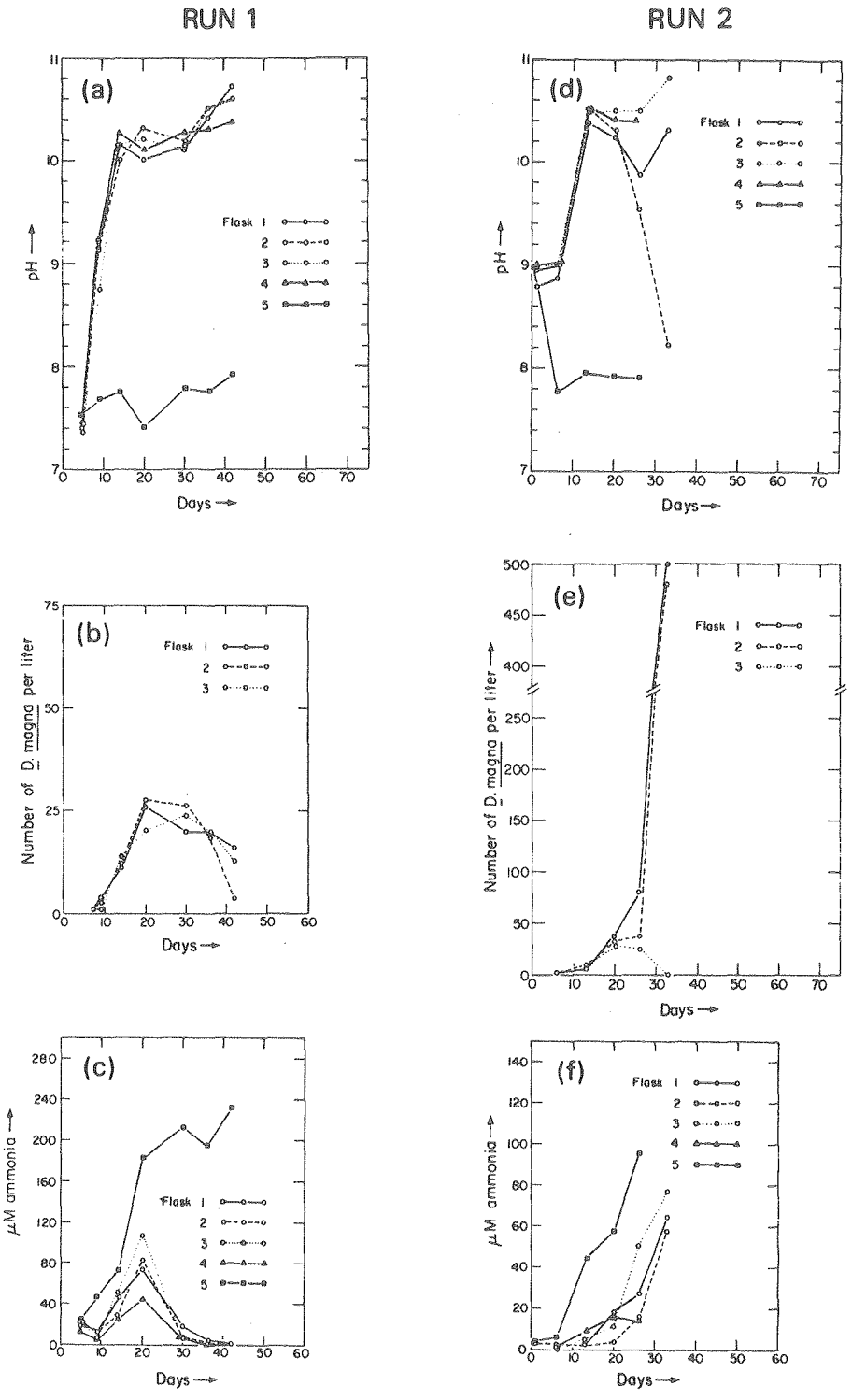

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Table 1. Algal and *Daphnia* media used in preliminary experiments.

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 Ankisdesmus; $NaHCO_3$ 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}