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New Diet for *Ceriodaphnia dubia*

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Ceriodaphnia dubia J. Richard 1894 (Berner 1986) has been sustained for the past three years on Ankistrodesmus convolutus Corda at the rate of about 20,000 cells per organism per day. To obtain adequate progeny for testing, daily feeding (7d/week) has been required. The purpose of this study was to develop a diet that would produce adequate progeny for testing and require feeding only three times a week. This paper describes this new diet.

MATERIALS AND METHODS

The conditions for culturing C. dubia have been previously published (Cowgill et al 1985a; 1985b; Takahashi et al 1987). This organism has been maintained in filtered (0.22 um) autoclaved Lake Huron water which was aerated 24 h prior to use. The mean chemical composition of this water has been previously published (Takahashi et al 1987). C. dubia has been sustained on A. convolutus in an axenic environment for about 5 years. This cladoceran was received under sterile conditions but the animals themselves were not bacteria-free.

C. dubia was maintained in an environmental growth chamber adjusted to 24°C. This chamber was devoted exclusively to rearing of animals. Neonates for testing the new diet were gathered by isolating gravid females in appropriate vessels 12 h prior to setting up tests and subsequently separating the two age classes using suitably sized sieves.

Two bacteria-free algal diets were developed to maintain C. dubia. The green alga A. convolutus was sustained in a medium designed by Provasoli and Pintner (1953) but with increased amounts of $NaNO_3$ and K_2HPO_4. Culturing conditions for A. convolutus have been previously described (Cowgill et al 1986). The second algal diet consisted of the diatom Nitzschia frustulum Kützing which was reared in ES-I-Si, a medium developed by Provasoli (1968) and revised here. The composition of six stock solutions which comprise this medium is shown in Table 1. Aliquots of these are added to double distilled water to produce a final stock solution A (cf Table 1). Two mL of solution A are sterilely added to

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Table 1. Composition of six stock solutions of medium ES-I-Sc.

Stock Solution		Grams/L*	Final Stock Solution A ML/L
I	B_{12}	0.1	1
	Biotin	0.05	
	Thiamine	5.00	
II	Na_2EDTA	10.0	25
	Fe (electrolytic)[1]	0.1	
	B (H_3BO_3)[2]	2.0	
	$MnCl_2 \cdot 4H_2O$	0.4	
	$ZnCl_2$	0.05	
	$CoCl_3 \cdot 6H_2O$	0.01	
III	$CuCl_2 \cdot 2H_2O$	0.25	1
IV	$NaNO_3$	175.0	
	Na_2 glycerophosphate	25.0	
	$FeCl_3 \cdot 6H_2O$	1.25	
V	Tris	200.0	25
VI	$Na_3SiO_3 \cdot 9H_2O$[3]	75.0	20

*Sterile filter (0.22 um) each stock solution before preparing solution A. Completed solution A must have a pH within the range 8.0 - 8.4 (HCl/KOH). Autoclave 30 min.

[1]Dissolve in HCl until no longer visible.

[2]Dissolve in H_2O with a few KOH pellets.

[3]Dissolve in pH 2 H_2O (HCl).

100 mL of filtered (0.22 μm) autoclaved Lake Huron water in a 250-mL Erlenmeyer flask covered with a Shimadzu closure. To this solution three other new supplements are added (Table 2). Culturing conditions for N. frustulum are shown in Table 3.

A. convolutus and N. frustulum were isolated from Linsley Pond, North Branford, Connecticut by K. Keating of Rutgers University, New Brunswick, New Jersey. C. dubia originated from Lake Superior, Minnesota, and was identified by D. Berner of Temple University, Philadelphia, Pennsylvania.

Table 2. Substances added to 100 mL filtered (0.22 μm) autoclaved Lake Huron water (under sterile conditions)

Substance			quantity/100 mL
Stock solution A			2 mL
Stock solution I			75 μL
Stock solution VII			75 μL
$FeCl_3 \cdot 6H_2O$	0.4840	g/L	
Na_2EDTA	10.0	g/L	
H_3BO_3	11.438	g/L	
$MnCl_2 \cdot 4H_2O$	1.441	g/L	
$ZnCl_2$	0.1042	g/L	
$COCl_3 \cdot 6H_2O$	0.0464	g/L	
Na_2SeO_4	0.0048	g/L	
$CuCl_2 \cdot 2H_2O$	0.0671	g/L	
Stock solution VIII			75μL
NaBr	12.878	g/L	
$SrCl_2 \cdot 6H_2O$	4.441	g/L	
RbCl	0.283	g/L	
LiCl	1.222	g/L	
$Na_2MoO_4 \cdot 2H_2O$	1.261	g/L	
Ki	0.013	g/L	

The testing conditions are identical to the culturing conditions. Each vessel contained 20 neonates at the initiation of the test. There were 2 control vessels and 3 test vessels. Controls were fed daily (7 days a week) a diet of *A. convolutus* 6.2×10^6 cells per culture vessel. The test vessels were fed once each on Monday and Wednesday and once in the morning and once in the late afternoon on Friday. Each feeding consisted of 9×10^6 cells per culture vessel of *A. convolutus* and 1.8×10^6 cells per culture vessel of *N. frustulum*.

RESULTS AND DISCUSSION

Table 4 shows the results for the feeding experiment. The first progeny arrived approximately 72 h after the initiation of the test. The difference between the mean of the control vessels and the mean of the test vessels is highly

Table 3. Culturing conditions for *N. frustulum*

Variable	Condition
Temperature (°C)	18.8 ± 0.3
Light intensity (lux)	395.6 ± 7.9
Photoperiod (h)	14 light/10 dark
pH: medium	8.23 ± 0.04
Habitat change	MWF
Inoculum size (cells/100 mL)	24.9×10^6
Agitation	Swirl flasks daily
Culture vessel mL	
Capacity	250
Content, Lake Huron H_2O	100
Medium	Revised Provasoli (1968)

Table 4. Results of a feeding experiment initiated with 20 neonates of *C. dubia* (First neonate arrived in ∿72 h).

	Total Progeny					
	Day 3	Day 4	Day 5	Day 6	Day 7	Day 8
Control	10	25	40	60	90	140
Control	10	26	47	58	88	145
Mean	10	25.5	43.5	59	89	142.5
Vessel-1	20	82	100	186	235	297
Vessel-2	25	87	110	185	240	305
Vessel-3	22	88	105	190	235	307
Mean	22.3	85.7	105	187	236.7	303
χ^2	4.68	32.6	25.5	66.6	67.0	57.8
P<	0.05	0.001	0.001	0.001	0.001	0.001
Controls	Daily *A. convolutus* 6.2×10^6 cells/culture vessel					
Vessels:	MWF (twice) *A. convolutus* + *N. frustulum*					
	A. convolutus 9×10^6 cells/culture vessel					
	N. frustulum 1.8×10^6 cells/culture vessel					

Table 5. Progeny per female obtained during feeding experiment

	Day					
	3	4	5	6	7	8
Control	0.5	1.3	2.0	3.0	4.5	7.0
Control	0.5	1.3	2.4	2.9	4.4	7.3
Mean	0.5	1.3	2.2	3.0	3.5	7.2
Vessel - 1	1.0	4.1	5.0	9.3	11.8	14.9
Vessel - 2	1.3	4.4	5.5	9.3	12.0	15.3
Vessel - 3	1.1	4.4	5.3	9.5	11.8	15.4
Mean	1.1	4.3	5.3	9.4	11.9	15.2

significant from Day 4 to the end of the test on Day 8, as measured by the chi-square test. Table 5 shows the mean brood size for each vessel.

Four months after this study was completed, another small study was initiated with 10 animals (all from brood 4-6), one per vessel. One animal never reproduced. The reproductive results of 9 animals for 14 days are shown in Table 6. It is clear from these results, that the new diet does an adequate job of sustaining C. dubia and encouraging the production of an adequate brood size. The variation in brood size depicted in Table 6 is pretty typical of C. dubia, and on this diet some of the animals produce broods daily, others will produce broods daily for 6 days and then every other day. C. dubia tends to produce larger broods when in groups (cf Table 5) than when reared alone.

Table 6. Mean brood size of nine animals after 4 months on the mixed algal diet

Brood Number	Mean Brood Size	Brood Number	Mean Brood Size
1	6.0	8	13.3
2	6.0	9	13.3
3	8.4	10	10.8
4	12.3	11	12.3
5	14.0	12	3.4
6	10.0	13	11.0
7	6.6	14	13.0

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